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COMMISSION DES COMMUNAUTÉS EUROPÉENNES

Direction Générale
DES AFFAIRES INDUSTRIELLES
TECHNOLOGIQUES ET SCIENTIFIQUES
Services de Biologie

ETUDE
DU METABOLISME DES DIFFÉRENTES CLASSES
D'IMMUNOGLOBULINES
CHEZ LA SOURIS NORMALE ET IRRADIÉE

H. BAZIN

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d'immunoglobulines chez la souris normale et
irradiée.

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J.F. HEREMANS.

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1. INTRODUCTION.

En 1908, Benjamin et Sluka montrèrent que, chez les lapins irradiés, les réactions immunitaires étaient d'une intensité notablement diminuée. Cette découverte attira l'attention des immunologistes, qui y virent essentiellement une possibilité de réaliser l'infection expérimentale d'animaux normalement réfractaires à certains germes. En revanche, les implications théoriques de ce travail ne suscitèrent que peu d'intérêt, car les connaissances immunologiques, à cette époque, n'étaient pas susceptibles d'apporter d'explication satisfaisante.

Ce n'est qu'après la deuxième guerre mondiale que la phase efficiente de ces études fut vraiment abordée. D'une part, les progrès, aussi bien théoriques que techniques, réalisés en radiobiologie, en immunologie et en biochimie, mirent à la disposition des chercheurs des moyens d'analyse beaucoup plus fins. D'autre part, les développements de l'énergie nucléaire, de la lutte contre le cancer et de la chirurgie de greffe, entraînèrent un regain d'intérêt que redoublait l'aspect fondamental de l'action des radiations ionisantes sur l'immunité et ses possibilités éventuelles d'applications pratiques.

L'étude de l'action des radiations ionisantes sur l'immunité peut être conçue soit en étudiant la réponse à un antigène particulier - et donc, la ou les catégories d'anticorps dirigés contre lui - en réponse à son injection, soit en considérant les fluctuations du taux sérique des diverses classes d'Ig prises comme des espèces chimiques.

Comme il était plus commode de doser une Ig en mesurant son activité-anticorps qu'en la caractérisant par ses propriétés physico-chimiques, on comprend aisément que la grande majorité des travaux furent accomplis par inoculation d'un antigène particulier suivie de la recherche des anticorps correspondants.

Toutefois, à de rares exceptions près (Hollingsworth, 1950; Taliaferro and Taliaferro, 1950; Perkins and Marcus, 1958), ces études négligèrent totalement le fait que le taux des anticorps sériques n'est que le reflet d'un équilibre entre la synthèse et le catabolisme de la ou des classes d'Ig dont ces anticorps font partie.

Il semblait donc intéressant, pour obtenir une vue plus cohérente de ce problème, d'aborder l'étude de l'action des radiations ionisantes sur les Ig non pas prises en tant qu'anticorps, mais uniquement en tant que molécules de protéines.

De cette manière, il est possible de connaître le sort collectif, classe par classe, d'anticorps préexistant à une irradiation, ou celui d'anticorps pouvant être synthétisés après une irradiation, soit en réponse primaire, soit en réponse secondaire.

2. ACTION D'UNE IRRADIATION DE COURTE DUREE SUR LES MAMMIFERES (Rappel).

Les mammifères ayant été soumis aux effets de radiations ionisantes extériorisent des symptômes variés en fonction de la dose reçue. Il est possible de distinguer trois grands syndromes dûs aux radiations ionisantes sur les mammifères. La littérature (Bond, Fliedner and Archambeau, 1965; Micklem and Loutit, 1966; van Bekkum and de Vries, 1967; van Bekkum, 1968) offre d'excellentes synthèses de ce sujet.

2.1. Le syndrome de "mort hématopoïétique" se manifeste pour des doses comprises entre 600 et 1200 Röntgens. La sévérité des symptômes est fonction de la dose. Il s'agit d'une inhibition plus ou moins complète de la prolifération normale des lignées cellulaires hémo- et lymphopoïétiques, les cellules sanguines périphériques disparaissant à une vitesse qui dépend de leur temps de maturation, puis de leur demi-vie. Les concentrations sanguines des granulocytes et des thrombocytes tendent vers un minimum, respectivement vers le 3^e-4^e jour et le 6^e-8^e jour après irradiation. Quant aux globules rouges sanguins, leur vie moyenne est beaucoup plus longue, environ 60 jours chez le rat et la souris, 120 jours chez l'homme. L'anémie mortelle qui peut survenir après irradiation est en fait plus précoce que ne l'indiquent ces valeurs (8-12 jours pour la souris), car elle est due à de multiples hémorragies. Dans le cas des cellules lymphoïdes, il existe en plus un effet direct des radiations sur celles-ci, qui contribue grandement à la sévère lymphopénie survenant dans les 24 premières heures après l'irradiation.

2.2. Le syndrome de "mort gastro-intestinale" est prédominant pour des doses comprises entre 1200 R et 12.000 R, l'organe cible étant le tractus gastro-intestinal. Chez la souris et le rat, la mort survient entre le 3^e et le 5^e jour; elle est vraisemblablement due à une violente perturbation des équilibres hydriques et minéraux associée à des pertes protéiques importantes.

Normalement, les cellules épithéliales de l'intestin, qui se multiplient dans les cryptes, migrent le long des villosités puis s'exfolient dans la lumière intestinale. Ainsi, chez la souris, le parcours d'une cellule le long d'une villosité prend 2 à 3 jours. Il a été montré que, quelques heures après une irradiation à dose convenable, un arrêt des mitoses dans la couche génératrice des cryptes intestinales, ainsi qu'une dégénérescence des cellules en cours de division, se produisent. Malgré un raccourcissement de la longueur des villosités, l'étalement des cellules épithéliales qui subsistent et l'augmentation de leur durée de vie, des solutions de continuité se créent dans la barrière intestinale, si nombreuses, dans certains cas, qu'elles peuvent entraîner la mort.

2.3. Pour des doses supérieures à environ 12.000 R, des signes caractéristiques de lésion du système nerveux général peuvent apparaître : convulsions, paralysie et stupeur. L'irradiation de la tête est suffisante pour entraîner la mort. Les lésions histopathologiques qui accompagnent ce syndrome sont des infiltrats granulocytaires périvasculaires et parenchymateux qui apparaissent dès la deuxième heure après l'irradiation; ils intéressent les méninges, les plexus choroïdes et le cerveau. Dans les 24 heures se développent des oedèmes et des lésions vasculaires du système nerveux central tout entier. La survie varie de quelques minutes à 1 à 2 jours.

Cette classification ne doit pas faire oublier de nombreuses autres causes importantes de mortalité telles que les troubles vasomoteurs (Haley, Andern, Riley and Williams, 1952) ou les infections. Ces dernières, en particulier, sont grandement favorisées par les troubles du système lymphoïde, et du tractus gastro-intestinal; les premiers ont une incidence directe sur les possibilités de défenses immunologiques des animaux irradiés, les seconds ouvrent la porte du milieu intérieur aux microorganismes du tube digestif.

3. IMMUNOGLOBULINES DE LA SOURIS (Rappel).

Depuis les travaux de Tiselius et Kabat (1939), il était pratiquement certain que la majorité des anticorps circulants de la souris, comme celle des autres mammifères supérieurs, se trouvait dans la fraction des γ -globulines de cet animal. Cependant, les immunoglobulines de la souris n'ont fait l'objet que de bien peu de recherches pendant de longues années. Il a fallu attendre cette dernière décennie pour que nos connaissances se développent en ce domaine. Grâce à l'immunoélectrophorèse, il a été possible de dénombrer de nombreux constituants sériques chez la souris (Grabar et Courcon, 1958; Heremans, Clausen, Heremans and Rask-Nielsen, 1959; Sassen, Kennes and Maisin, 1963). Au moyen de l'ultracentrifugation, Fahey et Humphrey (1962) mirent en évidence deux classes d'anticorps : 18S et 6,5S. Les myélomes à protéines monoclonales, étudiés tout d'abord par Potter, l'autorisèrent à distinguer deux classes d'Ig : système I et système II (Potter and Kuff, 1961), etc...

De cette façon, les diverses Ig sériques connues actuellement, furent inventoriées : les IgM furent distinguées des IgG (Fahey and Humphrey, 1962), de même, les IgA, des IgG (Fahey, 1961; Potter and Kuff, 1961) et les IgG₁, des IgG₂ (Merryman and Benacerraf, 1963). Ce sont pourtant, les publications de Fahey, Wunderlich et Mishell (1964 a et b) qui ont, pour la première fois, présenté des données d'ensemble claires sur les Ig de la souris.

Une grande confusion a malheureusement régné dans la nomenclature des Ig de la souris. De façon hâtive et sans beaucoup d'arguments, des classes d'Ig de cet animal ont été désignées de noms empruntés à la terminologie humaine. Le Tableau 1 donne la liste de ces principales appellations.

Tableau 1

Nomenclature des immunoglobulines de la souris

Bazin et Duplan, 1966. Warner, Herzenberg and Goldstein, 1966.	IgM ou γ M	IgA ou γ A	IgG ₁ ou γ G ₁	IgG _{2a} ou γ G _{2a}	IgG _{2b} ou γ G _{2b}
Potter and Kuff, 1961. Grabar, Kashkin et Courcon, 1963. Fahey, Wunderlich and Mishell, 1964. Dray, Potter and Lieberman, 1965. Potter, Lieberman and Dray, 1966.	β_2^M β_2^M ou γ_1^M γ M γ M	SII β_2^{II} β_2^A ou γ_1^A γ A γ A	β_3^{II} 7S- γ_1 γ F γ F	SI γ 7S- γ_{2a} γ G-Be 1 γ G	β_3^{III} 7S- γ_{2b} γ G-Be 2 γ H

Nous avons personnellement choisi la nomenclature de Fahey et coll. (1964 a et b) en l'adaptant aux règles énoncées par le Bulletin Mondial de la Santé (Ceppellini, 1964). La figure 1 représente schématiquement les diverses Ig de la souris telles qu'elles peuvent être observées au moyen de l'analyse immuno-électrophorétique.

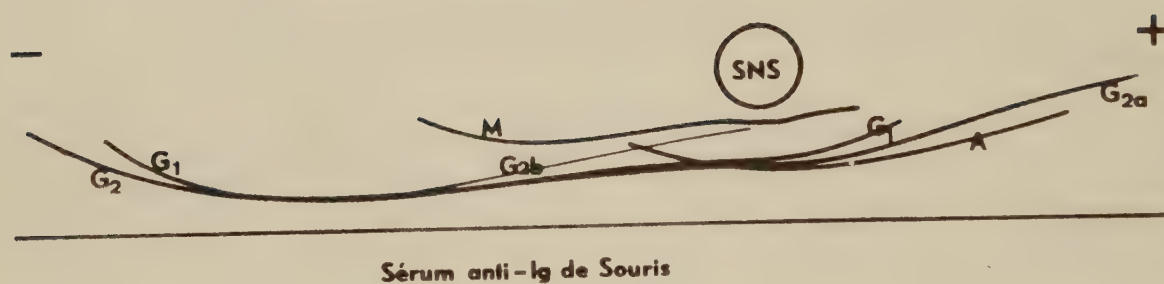


Figure 1. Diagramme d'une analyse immunoélectrophorétique d'un sérum de souris réalisé à l'aide d'un sérum de lapin anti-Ig de la souris (Bazin, 1966).

Le tableau 2 donne quelques propriétés physico-chimiques et biologiques des Ig de la souris (Bazin, 1967).

Tableau 2

Quelques propriétés physico-chimiques et biologiques
des immunoglobulines de la souris.

Références		Immunoglobulines				
Composition de la molécule		IgM	IgA	IgG ₁	IgG _{2a}	IgG _{2b}
		(2L+2H) [*] 5	(2L+2H) _n	(2L+2H)	(2L+2H)	(2L+2H)
Taux sérique normal (mg/ml)	Barth et coll.1965	0,6 à 2,5	0,2 à 3,6	0,9 à 2,4	0,8 à 5,7	—
	Warner et Wistar, 1968.	0,6 à 8,0	0,12 à 1,10	1,1 à 2,7	5,1 à 20,6	—
	Bazin et Doria, 1970c	4,33 ⁺ 0,10	0,53 ⁺ 0,02	1,42 ⁺ 0,04	12,94 ⁺ 0,40	—
Coefficient de sédimentation prédominant des molécules sériques	Fahey et coll.1964	18S	7S - 13S	7S	7S	7S
	Bazin et coll.1971b	—	9S	7S	7S	7S
Mobilité électrophorétique		β-γ rapide	α lente-β	β-γ	α lente-γ	β lente-γ
Demi-vie (jours)	Fahey and Sell, 1965	0,5	1	2,5	5,4	4,0
	Bazin and Malet, 1969	0,5	0,5	8 à 11	6 à 12	3 à 3,5
Locus allotypique	Herzenberg, McDevitt and Herzenberg, 1968	—	Ig-2	Ig-4	Ig-1	Ig-3

Suite du Tableau 2

Fixation du complément et propriétés lytiques	Ovary, Barth and Fahey, 1965	oui	non	non	oui	oui
Propriétés particulières			Existe partiellement dans les sécrétions	Se fixe sur les tissus de la même espèce, peut donner des phénomènes d'anaphylaxie locale	Se fixe sur les tissus d'espèces différentes, peut donner des phénomènes d'anaphylaxie passive locale	

*

H = chaîne lourde.

L = chaîne légère.

4. HISTORIQUE DU PROBLEME.

Ce n'est qu'avec l'introduction de l'électrophorèse par Tiselius (1937) que la véritable étude de l'influence des radiations ionisantes sur le taux des protéines sériques a commencé. Antérieurement, quelques travaux avaient été entrepris chez l'homme irradié à des fins thérapeutiques, ou chez le chien, soit en considérant le taux des protéines sériques totales, soit en les divisant en albumine et globuline (Hersfeld und Schinz, 1923; Wichels und Behrens, 1927; Breitländer und Lasch, 1927; Davy, 1931). Mais la faible proportion des immunoglobulines par rapport à l'ensemble des protéines du sérum n'a pu permettre de déceler aucune variation significative.

L'introduction de l'électrophorèse devait, en principe, autoriser des investigations bien plus précises dans ce domaine. Cependant, il faut remarquer que de nombreux travaux ne mirent en évidence aucun changement très significatif du taux des γ -globulines sériques chez le singe (Leone, Hartnett, Crist and McBeth, 1959), chez la chèvre (Waldschmidt, Leitz und Keller, 1961); chez le chien (Muntz, Gusman Barron and Prosser, 1949; Cornatzier, Engelstad, and Davidson, 1953; Goldwater and Etenman, 1957), chez le chat (Siminovic, Jankovic, Mihailovic and Isakovic, 1957), chez le loir (Schubert, Künkel and Maass, 1957), chez le lapin (Stender und Elbert, 1953; Meyniel et De Mende, 1955), chez le rat (Glinos and Berger, 1959; Erede and Podesta, 1961) et chez la souris (Shekarchi and Makinodan, 1957 et 1960; Bauer, Horowitz, Paronetto, Einherber, Abrams and Popper, 1964; Muramatsu, 1964).

La discordance de ces résultats avec les suivants provient vraisemblablement de ce que la méthode d'électrophorèse employée, la dose d'irradiation, l'état sanitaire des animaux d'expérience, enfin et surtout, l'espèce animale employée étaient des facteurs susceptibles d'influencer le taux réel ou apparent des γ -globulines. Le travail de Talmage, Freter et Taliaferro (1956), qui, chez le lapin, ont recherché une variation du taux des "globulines" par extraction au sulfate d'ammonium, fut également négatif.

Pourtant, d'autres auteurs ont trouvé une baisse plus ou moins marquée des γ -globulines après des doses subléthales et surtout léthales de radiations ionisantes.

Ce phénomène a été décrit dans plusieurs espèces animales : le lapin (Andreoni, Dompe et Russo, 1955); le rat (Westphal, Priest, Stets and Selden, 1953; Höhne, Künkel und Anger, 1955; Fisher, Magee and Coulter, 1955; Winkler und Paschke, 1955; Winkler und Paschke, 1956; Peters and Bauer, 1960; Sassen, Kennes et Maisin, 1963) et la souris (Werder, Hardin and Morgan, 1956; Werder, Hardin and Morgan, 1957; Mathé, Pays, Bourdon et Maroteaux, 1959; Amiel, Mathé et Pays, 1961). D'une façon générale, cette chute des γ -globulines paraît proportionnelle à la dose d'irradiation et au temps qui sépare le moment du prélèvement sanguin de celui de l'exposition aux radiations ionisantes, au moins dans les dix premiers jours. Il convient toutefois de remarquer que tous les animaux qui ont manifesté ces baisses de leurs taux sériques de γ -globuline sont des rongeurs.

Une étape importante fut ensuite franchie grâce à l'immunoélectrophorèse (Grabar et Williams, 1953) qui permit d'identifier de nombreux constituants sériques et en particulier les différentes immunoglobulines de la souris. Entre les mains de Grabar, Kashkin et Courcon (1963) cette technique montra que les différentes Ig disparaissent progressivement après une irradiation létale. La β_2 II (probablement l'IgA) et la β_2 M (ou maintenant l'IgM) ne purent plus être mises en évidence le quatrième jour après l'irradiation. La β_3 II et la β_3 III (probablement l'IgG₁ et l'IgG_{2b}), elles aussi, n'étaient plus visibles en immunoélectrophorèse très peu de temps après l'irradiation; en revanche, la ligne des γ -globulines s'effaça plus lentement dans le temps. Sassen, Kennes et Maisin (1966) ont repris ce travail chez le même animal. Ils ont trouvé que la β_2 II (IgA ?), après 900 R, disparaissait en 12 heures; la β_2 M, elle, avait complètement disparu 24 à 36 heures après l'irradiation. De même, la γ -globuline (IgG_{2a}), d'après leurs résultats, tombait à des valeurs de 10 à 15 % de la valeur normale, au moment de la mort des animaux irradiés. Enfin la β_3 II (IgG₁ ?) et la β_3 III (IgG_{2b} ?) leur semblaient diminuer moins vite que la γ -globuline.

Aucun de ces auteurs n'a cependant donné de résultats quantitatifs pour les différentes classes d'Ig. Après la mise au point d'une méthode de préparation d'antisérums spécifiques dirigés contre les différentes Ig de la souris (Bazin, 1966), il a été possible de reprendre cette étude de façon quantitative grâce à l'introduction de la technique de Mancini, Carbonara et Heremans (1965).

5. MATERIEL ET METHODES.

5.1. Souris.

Nous avons utilisé des souris mâles et femelles de diverses lignées pures : CBA/H, XVII, XVIIIE, C3H, C57/B1, GiftB ou des hybrides de première génération (C57/B1 femelles x C3H mâles) F_1 . Ces animaux avaient, en général, trois mois au moment de l'irradiation. La nourriture et l'eau ont toujours été fournies ad libitum pendant la durée des expériences.

5.2. Irradiations.

Les irradiations ont été effectuées dans plusieurs laboratoires et les dosimétries ne sont pas exactement comparables. Les doses léthales de nos diverses expériences (700 - 1000R) sont définies par une mortalité survenant du 9^e au 12^e jour après l'irradiation (mort hématopoïétique). Nous donnerons les diverses conditions avec chaque expérience.

5.3. Prélèvement sanguin.

Le sang a été prélevé au sinus rétro-orbital, au moyen de tubes de microhématocrite. Nous avons pris, en général, un ou deux de ces tubes par ponction. Le sérum a été centrifugé dans l'heure suivant le prélèvement et conservé en flacon de 0,8 ml, hermétiquement bouché, à -20°C.

5.4. Antisérums.

Nos antisérums ont été préparés, en grande majorité, à partir de lapins de trois kg. Certains, cependant, provenaient de cobayes. Les animaux ont été immunisés par deux ou trois injections intramusculaires ou intraplantaires. Lors de la première inoculation, l'antigène a été mélangé à de l'adjuvant complet de Freund. Lors des injections suivantes, il a été inoculé sans adjuvant. Deux à quatre semaines ont séparé la première injection des suivantes, qui ont été faites à deux jours d'intervalle.

Nous avons saigné nos lapins, à la carotide, et les cobayes, au coeur, sept à dix jours après la dernière inoculation.

Les diverses préparations antigéniques et les techniques d'absorption ont été publiées (Bazin, 1966; Bazin and Malet, 1969).

5.5. Diffusion en gélose.

5.5.1. Immunoélectrophorèse. Nous avons employé la technique de Grabar et Williams (1953) avec les modalités décrites par Heremans (1960).

5.5.2. Diffusion double en deux dimensions (Ouchterlony). Nous avons utilisé l'appareillage LKB (Suède) suivant les modalités conseillées par le constructeur.

5.5.3. Diffusion radiale simple (Mancini). Nous avons suivi la technique originale décrite par Mancini, Carbonara et Heremans (1965). Les titrages sont donnés généralement en mg/ml, par comparaison avec des solutions purifiées des diverses Ig de la souris (Bazin and Malet, 1969). Dans quelques cas, nous avons rapporté nos mesures aux valeurs d'un sérum de référence provenant d'un mélange de sérum d'environ cinq cents souris adultes de diverses lignées élevées dans des animaleries différentes. Les valeurs de chacune des classes d'Ig de ce mélange ont été prises arbitrairement égales à un.

6. RESULTATS.

6.1. Dose subléthale.

Nous avons étudié le taux sérique des Ig de douze souris XVII irradiées à une dose de 750 R (DL 30/30) (Bazin, 1968). Les conditions d'irradiation ont été décrites (Bazin and Malet, 1969). La moitié de ces animaux ont été irradiés in toto : les autres souris ont eu, au moment de l'exposition aux rayons X, les deux membres postérieurs protégés par du plomb. Les Figures 2 à 5 donnent les résultats de cette expérience. La chute de l'IgA a été très marquée entre le 6^e et le 8^e jour. Dans le cas des trois autres Ig, la dépression maximum a été observée au 10^e jour environ. La récupération d'un taux d'Ig comparable à celui du départ était assurée au 20^e jour et complète au 30^e jour. La protection des membres postérieurs n'a pas produit de différences notables entre les deux groupes expérimentaux. Cependant, dans le cas de l'IgM, il semble que la diminution du taux sérique ait été plus faible et que, dans le cas de l'IgA, la récupération d'un taux normal ait été plus rapide que chez les animaux complètement irradiés.

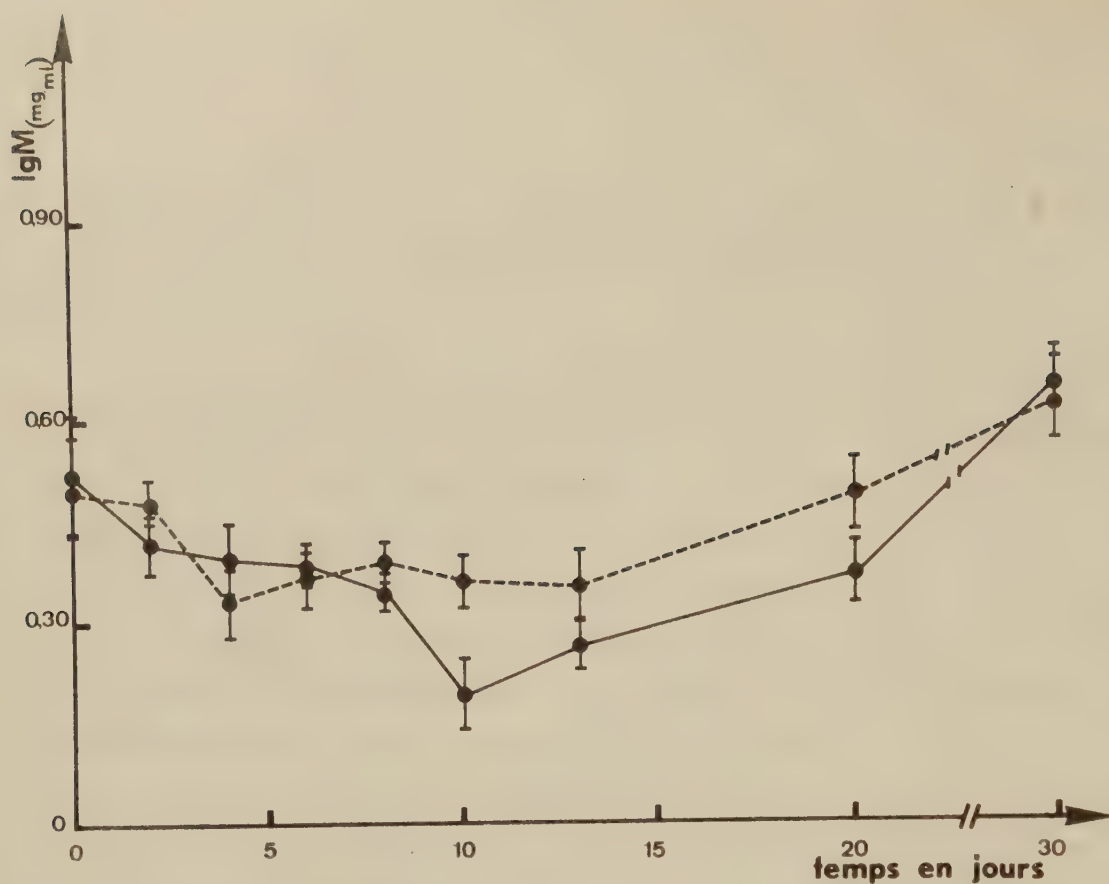


Figure 2. Concentration sérique de l'IgM en fonction du temps, chez des souris ayant reçu une dose de 750 R.

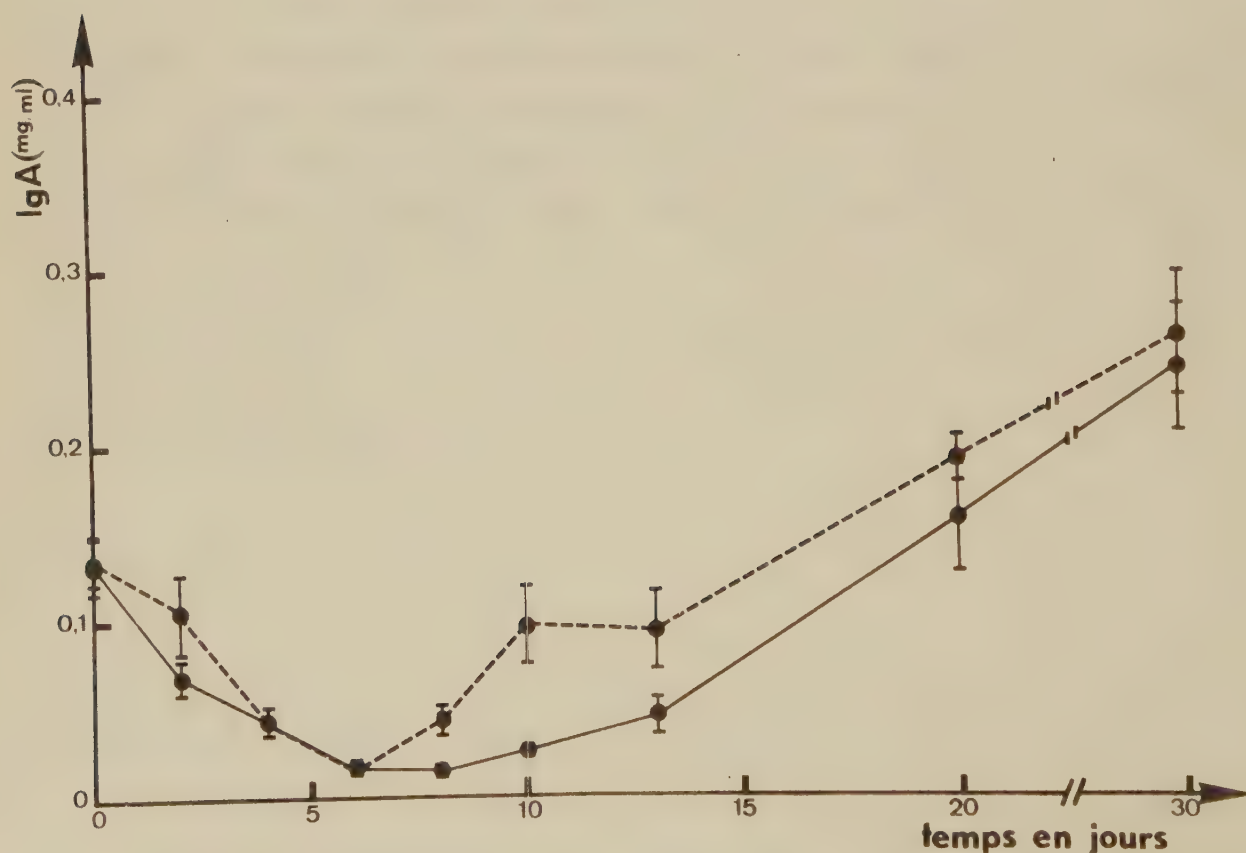


Figure 3. Concentration sérique de l'IgA en fonction du temps, chez des souris ayant reçu une dose de 750 R.

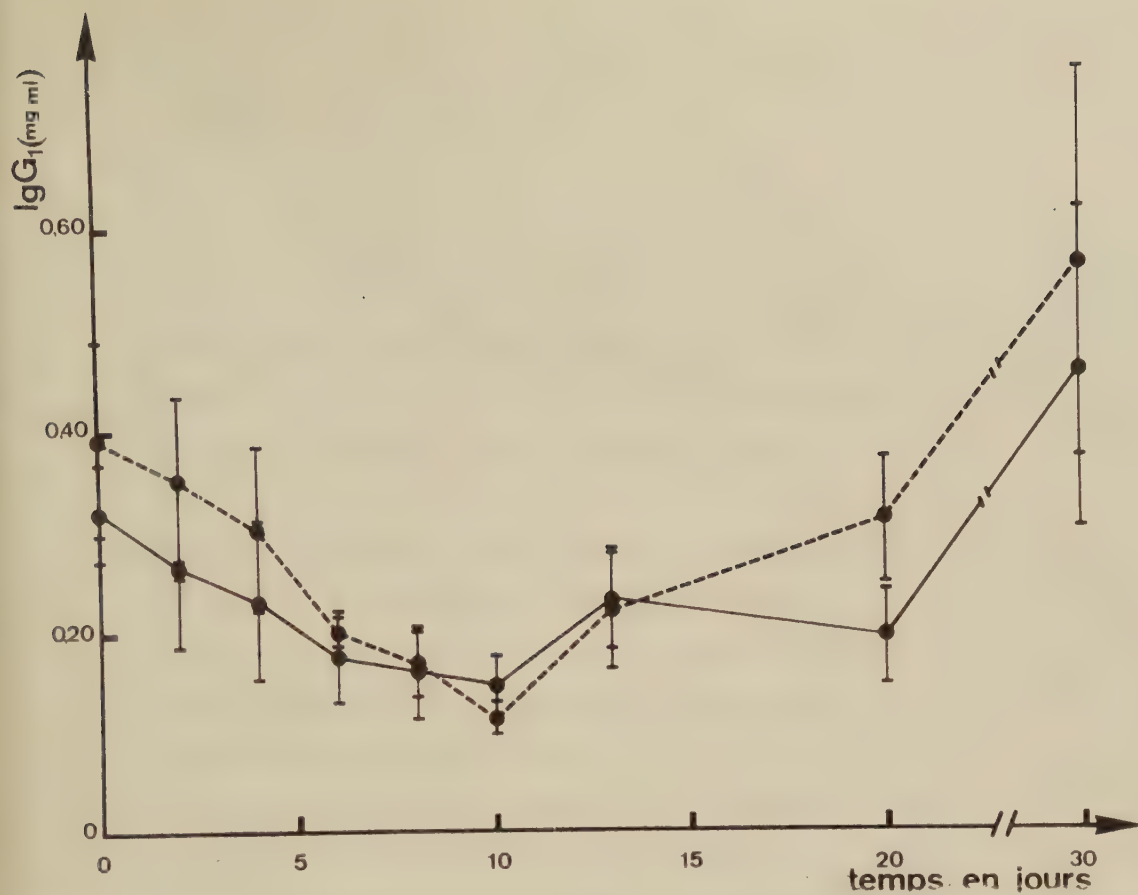


Figure 4. Concentration sérique de l'IgG₁ en fonction du temps, chez des souris ayant reçu une dose de 750 R.

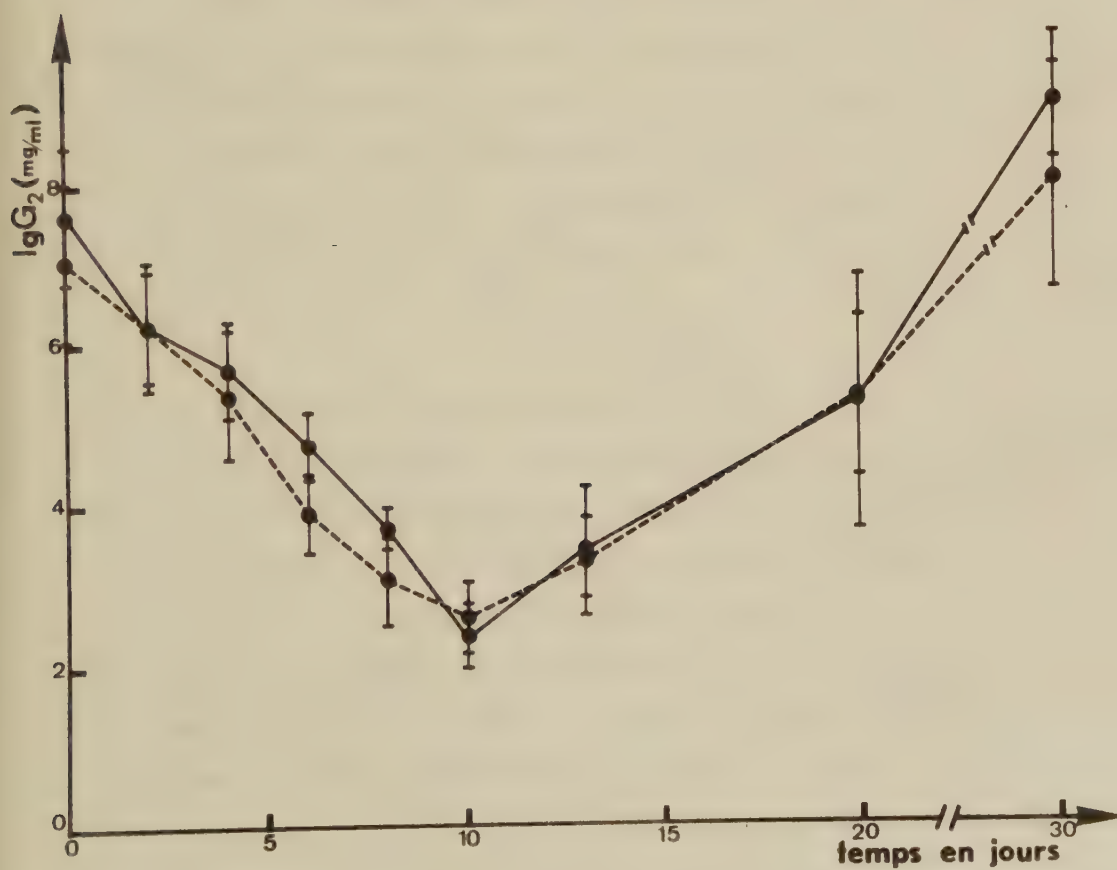


Figure 5. Concentration sérique de l'IgM en fonction du temps, chez des souris ayant reçu une dose de 750 R.

6.2. Dose létale.

Les souris irradiées à des doses létales (700-1000 R) mortes entre le 9^e et le 12^e jour après l'exposition aux rayons X, ont fait l'objet de la plus grande partie de ce travail.

Vingt souris CBA/H âgées de cinq mois ont été irradiées à des doses de 800 et 1000 R (conditions d'irradiation : 250 KV, 14 mA, épaisseur de demi absorption : 1,2 mm Cu). Les titrages des IgM, IgA, IgG₁ et IgG_{2a} donnèrent des résultats identiques pour les deux doses d'irradiation et les deux groupes ont donc été mélangés. La Figure 6 donne les pourcentages de ces quatre Ig, par rapport à la valeur de départ prise comme 100 %, aux jours 5, 7 et 9. Dans cette expérience, le taux sérique de l'IgM n'a baissé qu'à partir du 7^e jour environ, celui des diverses IgG a subi une diminution plus marquée et régulière dans le temps. L'IgA sérique, en revanche, a manifesté une chute très forte. Son niveau inférieur est apparu dès le 5^e jour et n'est pas remonté avant la mort des animaux d'expérience. Les différents pourcentages, au 9^e jour après l'irradiation, ont été, respectivement, de 65 %, 44 %, 33 % et 15 % pour les IgM, IgG_{2a}, IgG₁ et IgA (Bazin and Micklem, 1967).

Nous avons cherché à savoir si ce premier résultat expérimental dépendait de la souche de souris employée. A cet effet, six souris XVII ont été irradiées à une dose de 950 R (Bazin, 1968). La Figure 7 donne les résultats obtenus en pourcentage par rapport à la valeur de départ prise avant l'irradiation. Au dernier prélèvement avant la mort des animaux, il restait respectivement pour les IgM, IgA, IgG₁ et IgG_{2a} : 63 %, 24 %, 41 % et 54 %. De plus, la chute des IgA était encore plus précoce que celle des autres Ig.

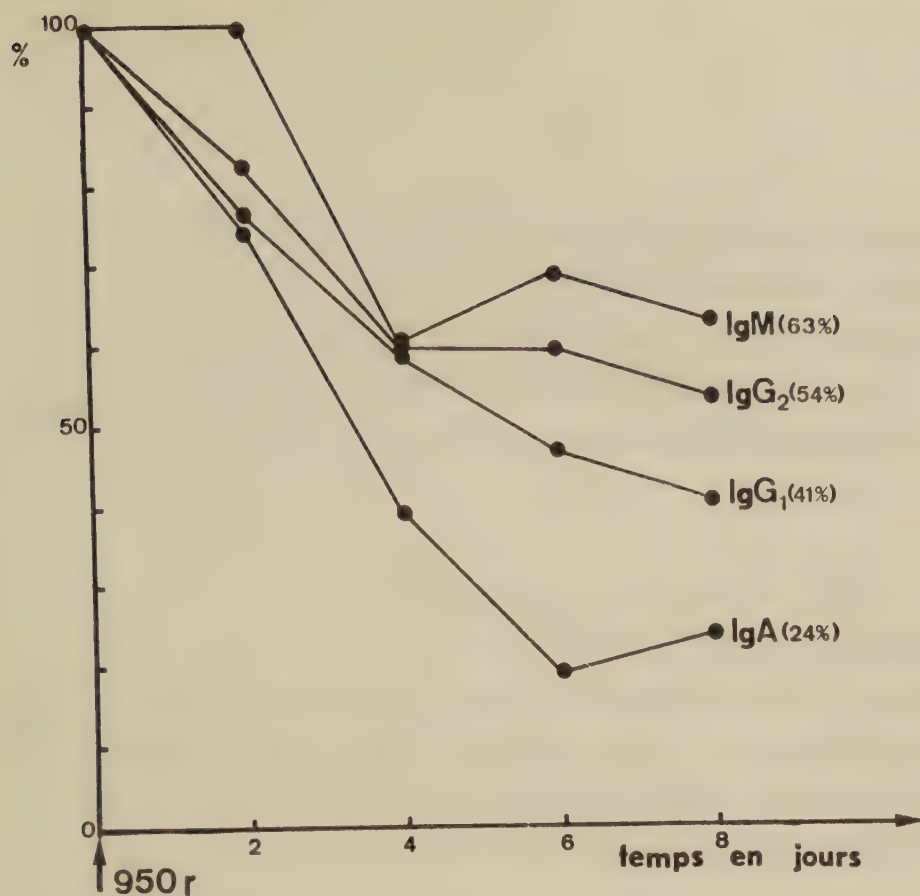


Figure 6. Concentration sérique des Ig, en fonction du temps, de 20 souris CBA irradiées à une dose létale de rayons X.

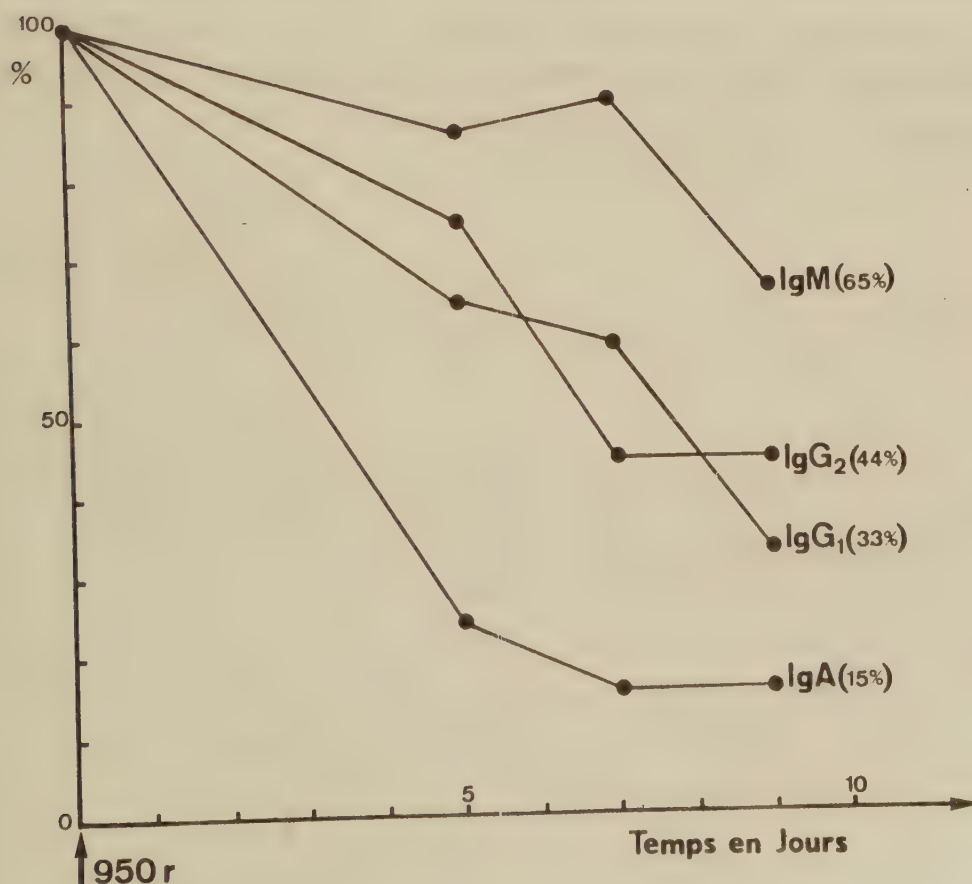


Figure 7. Concentration sérique des Ig, en fonction du temps, de 6 souris XVII, irradiées à une dose létale de rayons X.

Au cours de ces expériences, il a pu être vérifié que ces résultats étaient indépendants de la souche utilisée. En effet, des souris de souches Balb/c, C3H, GiftB et (C57/Bl femelles x C3H mâles) F_1 ont eu des modifications totalement comparables des taux sériques de leurs immunoglobulines après une irradiation léthale.

6.3. Dose supraléthale.

Les souris (C57/Bl femelles x C3H mâles) F_1 , âgées d'environ six mois, ont été irradiées à des doses de 1000, 1500, 2000, 3000, 4000 et 5000 R. Ce travail a fait l'objet d'une publication (Bazin and Doria, 1970c). La chute du taux sérique de l'IgM entre le jour 0 et le 3^e jour après l'irradiation s'est révélée faible et pratiquement identique pour toutes les doses. La baisse de l'IgA a été nettement plus prononcée, dès la 24^e heure après l'irradiation, pour les doses supérieures à 1000 R. La concentration sérique des deux IgG a décliné de façon très similaire et comparable à celle de l'IgM.

7. CATABOLISME DES IMMUNOGLOBULINES DE LA SOURIS NORMALE OU IRRADIEE.

Ce travail a fait l'objet de deux publications (Bazin, 1968; Bazin and Malet, 1969). Les souris employées étaient de souche XVII, normales ou irradiées à une dose de 950 R, dose 100 % létale, dans une période de 9 à 10 jours. Chaque expérience a été conduite en inoculant par voie intraveineuse une solution d'une des cinq Ig murines, marquées à l'iode 131 et provenant de sérum de souris normales ou de souris porteuses de myélome transplantable. La demi-vie de ces diverses Ig a été calculée en suivant la décroissance de la radioactivité de l'isotope radioactif soit dans le sérum, soit par comptage global du corps des animaux.

7.1. IgM.

Trois solutions différentes d'IgM ont été utilisées. La demi-vie de cette Ig, mesurée dans le sérum des animaux d'expérience, a été trouvée d'environ 12 heures aussi bien chez les souris normales que chez celles irradiées cinq jours avant ou 30 minutes après l'injection de l' ^{131}I -IgM.

7.2. IgA.

Cette étude a été conduite de façon similaire à celle de l'IgM. La protéine a été purifiée à partir de sérum de souris Balb/c porteuses du myélome EIII8. Après marquage à l'iode 131, elle a été inoculée, par voie intraveineuse, soit 30 minutes avant l'irradiation, soit cinq jours après. Aucune différence dans la vitesse du catabolisme sérique n'a pu être détectée au cours des trois essais différents. Les résultats montrent que la demi-vie sérique de l'IgA est d'environ 12 heures chez la souris normale; il en est de même chez la souris irradiée.

7.3. IgG.

Un mélange d'IgG₁, d'IgG_{2a} et d'IgG_{2b}, marquées à l'iode 131 et provenant de souris normales, a été employé dans cet essai. Ces trois IgG ont montré une demi-vie de 8,5 - 9 jours et de 4 jours, respectivement, chez les animaux normaux et irradiés.

7.4. IgG₁.

Deux préparations d'IgG₁ purifiées à partir des myélomes VIIA2 et MoPC/70A ont été utilisées au cours de trois expériences séparées. Par comptage de la radioactivité totale, la demi-vie trouvée pour cette Ig a été de 10 jours et de 6 jours chez les sujets normaux et irradiés, respectivement. En suivant la radioactivité sérique, la demi-vie d'une de ces préparations a été respectivement de 8 jours et de 4 jours, dans chacun des groupes.

7.5. IgG_{2a}.

Cette recherche a été effectuée à l'aide de la protéine myélomateuse extraite du sérum de souris porteuses du plasmocytome 5563. La demi-vie de cette Ig a été trouvée égale à 12 jours chez les sujets normaux et de 7 jours chez ceux irradiés, par comptage de la radioactivité globale. En étudiant la radioactivité sérique, nous avons trouvé des valeurs de 6 à 7 jours pour les souris normales et de 4 jours pour celles qui furent irradiées.

7.6. IgG_{2b}.

Deux préparations de la protéine monoclonale IgG_{2b} de l'immunocytome S188 ont été employées. Par comptage de la radioactivité sérique, nous avons obtenu une demi-vie de 3 à 3,5 jours et de 2 à 2,5 jours chez les souris normales ou irradiées, respectivement.

Ces expériences ont donc montré que le catabolisme de l'IgM et de l'IgA n'est guère influencé par une irradiation. En revanche, le catabolisme de l'IgG₁, de l'IgG_{2a} et de l'IgG_{2b}, étudié avec des protéines myélomateuses, s'est révélé significativement accéléré chez la souris irradiée. Un mélange d'IgG provenant d'animaux normaux a confirmé ce résultat.

8. ROLE DE L'INTESTIN.

Le tractus intestinal des mammifères a été décrit comme un site important de catabolisme de l'albumine (Birke, Likjedahl, Olhagen, Plantin and Ahlinder, 1963) aussi bien que des gamma-globulins (Andersen, Glenert and Wallevik, 1963). De même, il a été montré que la muqueuse intestinale de la souris possède une énorme population de plasmocytes qui contiennent de l'IgA (Crabbé, Bazin, Eyssen and Heremans, 1968a). Enfin, elle est extrêmement radio-sensible, comme chez tous les mammifères (Regaud, Nogier et Lacassagne, 1912).

Or Palmer et Sullivan (1959) ont prouvé que, chez le rat, une irradiation limitée à l'intestin est susceptible d'abaisser le taux sérique de l'albumine. De plus, Sullivan (1960) ainsi que Hornsey et Vatistas (1968) ont mis en évidence une excrétion massive de polyvinylpyrrolidone (PVP), un substitut des protéines plasmatiques, dans la lumière intestinale de la souris ou du rat irradiés.

Il était donc intéressant d'étudier le rôle particulier de l'intestin dans les changements métaboliques des Ig de la souris irradiée, ce qui a été fait soit en irradiant seulement l'intestin de certains animaux, soit en irradiant tout l'organisme d'autres animaux, à l'exception de leur tractus intestinal.

Ce travail a fait l'objet d'une publication (Bazin, Maldague and Heremans, 1970a). Des souris C3H âgées de 2 à 3 mois ont été utilisées. Dans tous les cas, l'intestin grêle, le caecum et une partie du colon ont été extériorisés après incision de la paroi intestinale. Au moment de l'irradiation, soit le tractus intestinal seul a été inclus dans le champ de l'appareil à rayons X, au moyen d'un localisateur cylindrique (Maldague, 1966), soit l'intestin, le caecum et une partie du colon ont été protégés en les plaçant dans une chambre de plomb spécialement aménagée à cet usage. Les doses d'irradiation ont été de 750 et de 700 R respectivement pour ces deux expériences, les titrages des diverses Ig étant effectués systématiquement au 5^e jour après l'irradiation.

Les résultats de ces expériences ont clairement indiqué que l'effet d'une irradiation totale sur la concentration de l'IgA sérique est dû aux lésions de l'intestin. L'irradiation exclusive de l'intestin a déterminé chez nos animaux d'expérience la décroissance caractéristique de l'IgA sérique, semblable à celle obtenue après irradiation "in toto". A l'inverse, la seule protection de cet organe l'a évitée.

Le taux de l'IgM sérique, en revanche, n'a pas varié après une irradiation intestinale. Dans le cas de l'irradiation totale avec protection de l'intestin, ce taux a seulement subi un déclin modéré.

Enfin les taux sériques des IgG₁ et des IgG₂ se sont révélés plus influençables à une irradiation de l'intestin que celui de l'IgM. Au contraire, l'irradiation de tout l'organisme, associée à la protection du tractus intestinal, n'a apporté que peu de changement dans le taux de ces deux Ig.

Il faut remarquer que ces résultats ont été déterminés au 5^e jour après l'irradiation, c'est-à-dire, à un moment où le taux sérique de l'IgM, en particulier, n'a encore que très peu varié chez les souris irradiées in toto, à une dose comparable.

9. SYNTHESE DU TRAVAIL.

Il est certain que le taux sérique d'une immunoglobuline est le résultat de deux actions agissant en sens opposé : d'une part l'anabolisme qui tend à l'augmenter, d'autre part le catabolisme qui tend à l'abaisser.

Il a été démontré que la synthèse des protéines dans une cellule ayant subi une irradiation de l'ordre de 500 à 1000 R, était peu affectée et même, parfois, augmentée (Cammarano, 1962). En particulier, ceci paraît être le cas des immunoglobulines étudiées en tant que γ -globulines (Reuter, Sassen and Kennes, 1967; Bazin, 1968) ou en tant qu'anticorps (Kennedy, Till, Siminovitch and McCulloch, 1969). L'étude, par immunofluorescence, du nombre de plasmocytes des différentes classes d'immunoglobulines, chez la souris préalablement irradiée par une dose de 700 R n'a pas non plus révélé de modifications perceptibles de leur nombre, dans les ganglions mésentériques et périphériques ou dans la rate (Bazin et coll., 1971b).

L'analyse du catabolisme de ces diverses classes d'immunoglobulines a montré, par contre, une nette différence de comportement entre les IgM et les IgA, dont le catabolisme sérique ne se modifie pas après une irradiation, et les diverses IgG dont le catabolisme sérique est nettement augmenté.

Il est possible de diviser en trois groupes les cinq classes d'immunoglobulines de la souris en fonction de l'action des radiations ionisantes sur elles.

9.1. IgM.

Dans les différentes classes d'immunoglobulines, le taux sérique de l'IgM est le moins perturbé par les effets des rayons X. Peu d'études ont été consacrées directement à l'IgM. Cependant beaucoup de travaux effectués sur l'action des radiations ionisantes sur les réponses immunologiques ont pris comme modèle la formation des hémolysines après inoculation de globules rouges hétérologues (Taliaferro, Taliaferro and Jaroslow, 1964; Simic, Sljivic, Petrovic et Cirkovic, 1965; Sado, 1968). Il ressort de ces expériences que les réactions immunologiques déjà enclenchées avant l'irradiation ne sont que très peu troublées par les radiations ionisantes. En revanche, l'irradiation préalable à l'injection antigénique est fortement suppressive, ce qui s'explique par la radiosensibilité des immunocytes en voie de division (Kennedy et coll., 1965).

Il est probable que la légère baisse des immunoglobulines M, après irradiation, peut être attribuée d'une part à l'interruption de la multiplication des immunocytes de ce type, d'autre part, à un effet faible mais possible, sur le catabolisme de ces immunoglobulines, effet difficile à mettre en évidence par suite de la demi-vie très brève de l'IgM.

9.2. IgA.

Chez la souris, c'est l'immunoglobuline dont le taux sérique est le plus sensible à une irradiation. Sa chute, déjà nette pour une dose subléthale (Bazin, 1968), est dramatique après une dose supraléthale (Bazin and Doria, 1970c). Deux raisons principales pouvaient rendre compte de cette baisse du taux sérique de l'IgA après irradiation : la perte sélective d'IgA sérique dans la lumière intestinale, ou une perturbation du mécanisme de son approvisionnement sérique. Ces dérangements

pouvaient être déclenchés par les effets d'une irradiation restreinte à l'intestin (Bazin et coll., 1970a). La première était improbable car la demi-vie de l'IgA sérique, marquée à l'iode radioactif, n'était pas raccourcie par une irradiation. Il fut possible de renforcer cette conclusion en montrant que le taux de l'IgA circulante n'était pas abaissé, après une irradiation, si son système d'approvisionnement sérique n'était pas lésé (Bazin et coll., 1971a). Dans une première expérience, par la greffe sous-cutanée d'un plasmocytome, une quantité très élevée d'IgA d'origine extraintestinale a été fournie au système circulatoire des animaux testés. L'irradiation d'une partie de ces souris n'a pas produit de chute de leur IgA sérique. Dans une deuxième expérience, des souris axéniques à taux sérique d'IgA naturelle extrêmement bas, furent transfusées avec du sérum normal de souris. Ici encore, le catabolisme de cette IgA sérique n'a pas été modifié du fait d'une irradiation.

Il était donc nécessaire de rechercher dans un défaut d'approvisionnement de l'IgA sérique, la chute spectaculaire de cette Ig, consécutive à une irradiation. Deux possibilités étaient encore à envisager : soit une radio-sensibilité nettement plus grande des plasmocytes à IgA vis-à-vis des autres plasmocytes, soit un détournement de l'IgA normalement destinée au système circulatoire, vers une autre destinée.

Il a déjà été démontré que les mécanismes de synthèse des protéines et des immunoglobulines en particulier sont peu radiosensibles. En fait, pour des doses d'irradiation conduisant à une "mort hématopoïétique", les effets des radiations ionisantes sur les cellules en phase de synthèse d'Ig sont probablement indétectables au niveau du taux sérique de l'Ig en question.

Cependant on a trouvé une diminution nette mais modérée des plasmocytes intestinaux, après une irradiation de 700 R. Cette relative radiosensibilité semblait dépendre de l'environnement de ces cellules plutôt que d'une sensibilité qui leur seraient propre, car les plasmocytes à IgA paraissent se comporter identiquement aux autres plasmocytes, dès que leur étude est considérée organe par organe. On peut avancer que les plasmocytes intestinaux pourraient être particulièrement exposés aux substances libérées par les cellules épithéliales en voie de dégénérescence, ainsi qu'à des rentrées d'endotoxines provenant de la lumière intestinale.

Plus précisément, des résultats expérimentaux quantitatifs obtenus à l'aide de culture "in vitro" d'intestin de souris normales ou irradiées, ont montré qu'une éventuelle diminution de synthèse de l'IgA intestinale ne pouvait pas expliquer l'ampleur de la chute de concentration de cette Ig après une irradiation. En effet, la biosynthèse minimum, obtenue au 4^e jour après 700 R, était encore de 56 % de sa valeur normale (Bazin et coll., 1971b).

Il était donc assuré que la chute du taux d'IgA circulante de la souris irradiée ne correspondait ni à une augmentation de son catabolisme sérique, ni à une diminution importante de sa biosynthèse intestinale. De plus il était certain que l'IgA sérique de la souris provenait en grande partie des nombreux plasmocytes à IgA de l'intestin (Bazin et coll., 1970a). Enfin il avait été démontré qu'un pourcentage très important de l'IgA trouvée dans les sécrétions intestinales de cet animal provient du chorion de sa muqueuse intestinale (Crabbé et coll., 1968b; Bazin et coll., 1971b). Deux voies d'évacuation sont donc offertes normalement à l'IgA synthétisée dans la muqueuse intestinale : d'une part, les vaisseaux lymphatiques puis sanguins, d'autre part, la lumière du tube digestif après passage à travers l'épithélium intestinal. Dans les jours

qui suivent une irradiation létale, il a été trouvé que la quantité absolue d'IgA excrétée dans l'intestin n'était pas sensiblement modifiée, quoique sa synthèse subisse une diminution d'un tiers environ. Il devenait probable que l'irradiation favorisait grandement la sortie de l'IgA vers l'intestin au détriment de l'approvisionnement du système circulatoire. L'étude de l'épithélium intestinal après irradiation a fourni un appui morphologique à cette hypothèse (Bazin et coll., 1971b). En effet, la grande radiosensibilité des cellules-souches de cet épithélium conduit à une raréfaction des cellules épithéliales le long des villosités intestinales. Il en résulte de graves lésions et une augmentation de la perméabilité de la barrière épithéliale intestinale.

Chez la souris normale, les espaces intercellulaires sont extrêmement étroits; de la membrane basale jusqu'à la bordure en brosse; ils sont oblitérés de place en place, d'abord, par une série de desmosomes, puis par la barre terminale. Chez la souris irradiée, dès le premier jour après l'exposition aux radiations ionisantes, il a été possible d'observer des décollements des parois cellulaires qui vont en augmentant, présentent un maximum d'intensité au 3^e jour, et régressent ensuite, tout en restant encore présents pendant la phase de restauration de l'épithélium.

Il y a peu de doute que ce système de canalicules offre à l'IgA une voie de sortie particulièrement aisée, en augmentant la fraction de cette immunoglobuline, qui, normalement, est évacuée dans la lumière intestinale, au préjudice de celle qui emprunte les voies lymphatiques pour rejoindre le système sanguin.

9.3. IgG.

Trois classes d'immunoglobulines de type G ont été décrites chez la souris. Après une irradiation, leurs taux sériques paraissent avoir un comportement identique, c'est-à-dire une chute relativement importante d'au moins 50 pour cent chez des animaux qui succombent au syndrome hématopoïétique.

- D'une part, il est établi que le taux de synthèse des IgG, après une irradiation léthale, est peu modifié (Reuter et coll., 1967; Bazin, 1968). (L'analyse par immunofluorescence du nombre des plasmocytes à IgG dans la rate et les ganglions lymphatiques de la souris irradiée a d'ailleurs corroboré ces résultats).

- D'autre part, le catabolisme des IgG s'est montré accéléré, aussi bien dans le compartiment vasculaire seul, que dans les compartiments extra- et intravasculaires étudiés simultanément (Bazin, 1968; Bazin et Malet, 1969). De plus, une irradiation localisée à l'intestin a permis de constater qu'il était suffisant de léser ce seul organe pour obtenir une chute significative de ces IgG (Bazin et coll., 1970a), bien que le nombre de plasmocytes intestinaux de ce type soit presque négligeable (Bazin et coll., 1971b).

Grâce à la collaboration du Dr. J.P. Thierry, il a été possible d'étudier le problème du catabolisme intestinal des IgG chez des souris XVII E, âgées d'environ trois mois. On a soumis une partie de ces animaux à une irradiation de 950 R suivant la technique déjà décrite (Bazin, 1968). Pour essayer de rendre visible le cheminement des molécules des vaisseaux sanguins vers la lumière intestinale, trois marqueurs ont été employés, repérables en microscopie électronique et de dimensions encadrant celle des immunoglobulines IgG : peroxydase de radis (CALBIOCHEM), ferritine de cheval (CALBIOCHEM), et Thorothrast (Fellows-Testagar, Detroit, U.S.A.).

La première de ces substances, d'un poids moléculaire de 40.000, a été employée après une dialyse intensive contre de l'eau physiologique pour éviter la dégranulation des mastocytes et les oedèmes qui accompagnent l'injection des préparations commerciales. A l'inverse de ce premier marqueur de dimensions inférieures aux molécules d'immunoglobulines IgG, les deux autres marqueurs employés étaient de taille supérieure : la ferritine de cheval, de poids moléculaire d'environ 600.000 et de diamètre moyen de 100 Å, le thorothrast (dioxyde de thorium en suspension colloïdale) de taille particulière variable allant de 50 à 1000 Å avec une moyenne comprise entre 50 et 100 Å. La ferritine de cheval a été utilisée après une dialyse contre de l'eau physiologique additionnée d'EDTA pour enlever le cadmium de cristallisation qui, inoculé à la souris, produit un effet vasoconstricteur et toxique.

Après des temps variables allant de un à cinq jours après l'irradiation ou son simulacre, un des trois marqueurs a été injecté par voie intraveineuse à l'une des vingt-neuf souris irradiées ou des neuf souris normales employées dans cette expérience. L'animal était anesthésié et sa cavité abdominale ouverte pour pouvoir dégager une anse intestinale du jejunum proche du duodénum. Celle-ci était alors obturée à chacune de ses extrémités ainsi que les vaisseaux sanguins y conduisant. On procédait ensuite, après des temps variables (deux minutes et demi ou six minutes), à la fixation de l'anse en introduisant de la glutaraldéhyde à 4 %, à faible pression dans sa lumière et en l'aspergeant de cette même substance. Les autres détails de la technique employée ont déjà été décrits (Bazin et coll., 1971b).

9.3.1. Chez l'animal normal, il est possible de trouver dans de rares cellules (entérocytes ou macrophages) quelques agrégats de molécules de ferritine dans des inclusions cytoplasmiques, mais jamais dans un espace intercellulaire ou dans les vaisseaux sanguins. L'aspect morphologique des particules de thorothrast ne prête à aucune confusion. Enfin on ne trouve de structures ayant une activité peroxydasique que dans les leucocytes.

9.3.2. Chez l'animal non irradié, inoculé par voie intraveineuse avec l'un des trois marqueurs, deux de ceux-ci, la ferritine et le thoro-thrast, restent localisés dans la lumière des capillaires. Il est remarquable cependant que certains vaisseaux sanguins n'en contiennent pas. Il n'y a pratiquement pas de passage extravasculaire de ces marqueurs, même après six minutes. En revanche, pour la peroxydase, il y a un passage lent, mais faible, visible déjà au bout de trois minutes après l'injection, et dû à un mécanisme de pinocytose.

9.3.3. Chez l'animal irradié et injecté avec l'eau physiologique (NaCl 9 %) on retrouve les lésions épithéliales déjà décrites (Bazin et coll., 1971b).

9.3.4. Chez l'animal irradié et inoculé avec de la ferritine de cheval ou du thoro-thrast, il est possible d'identifier les marqueurs dans les capillaires et dans les espaces conjonctifs périvasculaires. Le passage à travers l'endothélium vasculaire semble se faire par le biais de deux mécanismes différents. D'une part le phénomène de pinocytose assure un passage dont l'importance paraît faible et peu sensible à l'irradiation; d'autre part, un cheminement des traceurs se fait au niveau de l'affrontement des cellules endothéliales contiguës. Ce phénomène, inexistant chez l'animal normal, est à considérer comme pathologique et traduit des lésions de l'endothélium vasculaire. Quelques particules de ces traceurs étant même retrouvées au pôle basal des entérocytes, la communication est possible à travers les cellules épithéliales des vaisseaux et des basales jusqu'au niveau des entérocytes.

La peroxydase de radis est visible en grande quantité dans la majorité des capillaires sanguins des souris irradiées, inoculées avec cette substance. Dans le cas de cette petite molécule, la sortie semble plus importante par pinocytose qu'avec les deux autres marqueurs. De plus et surtout, il existe des communications aisées entre la lumière des capillaires et les espaces conjonctifs périvasculaires au niveau des jonctions inter-cellulaires endothéliales des vaisseaux. Il est aisé de suivre le cheminement de la peroxydase hors des vaisseaux, puis dans les espaces parfois très grands qui séparent les entérocytes, même dans des portions de l'épithélium intestinal qui semble intact à un examen histologique optique. La barre terminale reste cependant toujours en place et obture la voie de communication. Il est vraisemblable, qu'arrivées à ce niveau, les molécules sont obligées de passer à travers le pôle apical des entérocytes pour pouvoir être rejetées dans la lumière intestinale.

Manifestement, il y a deux barrières susceptibles d'arrêter les molécules d'immunoglobulines IgG au niveau de l'intestin : d'une part, l'endothélium des capillaires sanguins, d'autre part l'épithélium intestinal.

L'étude du catabolisme sérique des trois groupes d'immunoglobulines de la souris nous a montré que seule l'IgG avait son catabolisme intestinal augmenté de façon marquée après une irradiation (Bazin et Malet, 1969; Bazin et coll., 1970a). Or les molécules d'IgA qu'il est possible de déceler au niveau de la lamina propria sont en majorité d'un poids moléculaire identique à celui des IgG (Bazin et coll., 1971b) et peuvent assez aisément traverser la barrière épithéliale pour se retrouver dans la lumière intestinale (Bazin et coll., 1971b). Il semble que le facteur limitant de la perte des IgG au niveau de l'intestin est la perméabilité de l'endothélium vasculaire lequel filtre les molécules susceptibles

de sortir des capillaires, en fonction de leur taille. Il y a peu de passage de grosses molécules ou d'agréats comme dans le cas de la ferritine ou du thorothrast et peu de passage aussi pour des molécules d'IgM ou d'IgA sérique 9-11S (Bazin et coll., 1971b; Bazin et Malet, 1969); par contre, un passage appréciable de molécules d'immunoglobulines IgG et de véritables pertes en cas de petites molécules comme la peroxydase.

Il est donc probable que, chez la souris, la chute des IgG après irradiation est due à une diminution de leur synthèse, comparable à celle de toutes les Ig et en particulier de l'IgM, accompagnée d'un accroissement du catabolisme intestinal provoqué par l'association de lésions de l'endothélium des capillaires sanguins et de l'épithélium intestinal.

10. IMPLICATION DE CE TRAVAIL DANS LA PATHOLOGIE DES SOURIS IRRADIEES.

L'importance des infections bactériennes ou virales dans la mortalité due aux irradiations, a fait l'objet de longues et nombreuses controverses. Il est bien certain que les trois grands syndromes : hématopoïétique, gastro-intestinal et nerveux peuvent expliquer la plupart des morts consécutives à l'exposition aux radiations ionisantes. Il n'empêche qu'après une irradiation, de profondes différences existent entre les résultats obtenus, aussi bien pour ce qui concerne le pourcentage de mortalité que le temps de latence à partir de sujets d'une même espèce entretenus dans des conditions sanitaires différentes. Il est non moins évident que la voie d'invasion la plus aisée, créée par les radiations ionisantes, est la voie intestinale : car d'une part, l'endothélium à ce niveau, est unicellulaire et très radiosensible du fait de sa vitesse élevée de renouvellement, d'autre part le tube digestif contient un milieu extrêmement septique.

Le système immunologique intestinal est fort développé. Il est représenté par des organes lymphoïdes localisés en certains points (formations lymphoïdes naso-pharyngées-plaques de Peyer-appendice) et par une multitude de cellules lymphoplasmocytaires qui paraissent remplir l'espace sous-muqueux de la totalité du tube digestif, formant une sorte de manchon protecteur. On doit à Crabbé, Carbonara et Heremans (1965) d'une part, et à Rubin, Fauci, Sleisenger et Jeffries (1965) d'autre part, la découverte de l'extrême richesse en plasmocytes à IgA de la sous-muqueuse intestinale de l'homme, fait retrouvé chez d'autres espèces animales, en particulier chez la souris (Crabbé et coll, 1968a).

En outre, ces mêmes auteurs ont montré que, chez la souris axénique, les immunocytes sont très peu nombreux. Cependant l'exposition à l'environnement normal fait rapidement apparaître, chez ces animaux, une population normale de plasmocytes intestinaux (Crabbé, Nash, Bazin, Eyssen and Heremans, 1970).

Dans la lutte contre les infections intestinales, l'immunité humorale peut offrir deux lignes principales de résistance : intestinale, puis sanguine.

Les sécrétions intestinales ont depuis longtemps été étudiées pour l'activité biologique de leurs copro-anticorps (Davies, 1922). Burrows et coll. démontrèrent en 1947 qu'il est possible de mettre en évidence des agglutinines anti-Vibrio cholerae dans les faeces de cobaye auxquels ce germe avait été administré par voie gastrique. Dans ce cas, l'évolution du taux de ces copro-anticorps précédait celle des anticorps sériques. Par contre, en cas d'inoculation parentérale, les anticorps sériques et les copro-anticorps apparaissaient, culminaient et disparaissaient simultanément. De très nombreux exemples de réponse immunologique attribuable à la muqueuse intestinale ont été mis en évidence pour des antigènes allant de la protéine soluble aux parasites confinés au tube digestif (Bazin, 1970a,c). La classe de ces copro-anticorps n'a été que rarement déterminée. Cependant chez la souris (Crabbé et coll., 1968b) comme chez l'homme (Chodirker and Tomasi, 1963), le pourcentage d'IgA par rapport aux autres Ig dans les sécrétions intestinales est nettement supérieur à ce même rapport étudié dans le sérum. La majorité de cette IgA est d'origine locale (Crabbé et coll., 1968b; Bazin et coll., 1971b). Les IgM et les IgG, en revanche, proviennent du sérum car les nombres des plasmocytes intestinaux des deux derniers types sont peu élevés (Bazin et coll., 1971b).

Une autre ligne de défense de l'organisme contre les infections d'origine intestinale, est située dans les vaisseaux lymphatiques ou sanguins. Là encore, si les IgM et les IgG sont d'origine générale, l'IgA provient en grande partie du manchon plasmocytaire intestinal chez la souris (Bazin et coll., 1970a); il en est de même chez le chien (Vaerman and Heremans, 1970).

La stimulation pérorale d'un animal semble conduire à deux types de réponses immunologiques : d'une part des réponses générales de type IgM-IgG, d'autre part, des réponses locales à prédominance IgA (Heremans, 1969). Dans le premier cas, l'absorption rapide, puis le passage de l'antigène dans les vaisseaux chylifères, correspond en fait à une injection intraveineuse. Dans le deuxième cas, l'antigène semble être absorbé lentement et pourrait rester en contact avec le tissu lymphoïde intestinal. Des expériences réalisées chez la souris axénique, avec la ferritine comme antigène, ont montré, dans ce système du moins, que la réponse immunologique était presque entièrement circonscrite aux plasmocytes intestinaux et à la classe des IgA (Crabbé, Nash, Bazin, Eyssen and Heremans, 1969). D'autres études conduites avec des globules rouges de mouton donnés dans l'eau de boisson de souris axéniques, ont prouvé que des réponses immunologiques d'origine intestinale pouvaient être décelées au niveau des organes lymphoïdes extraintestinaux, dans toutes les classes d'immunoglobulines avec, cependant, une nette prédominance du type IgA (Bazin, Levi and Doria, 1970b). De même, les anticorps circulants formés après l'administration orale d'un antigène semblent être en rapport avec le mode d'absorption de ce dernier : une réponse de type entérique donne une contribution majoritaire de la classe IgA (Heremans, 1969).

Il semble raisonnable de supposer que les très nombreux plasmocytes à IgA qui entourent la cavité intestinale, contribuent à interdire à la flore intestinale tout accès à l'organisme. Chez la souris, une irradiation léthale conduit, dans l'intestin, à une baisse du taux de synthèse de l'IgA, corrélativement à une légère diminution du nombre des plasmocytes. Parallèlement, il se produit une chute prononcée et rapide de l'IgA sérique. Il est probable que ces troubles sélectifs du système immunologique à IgA, après irradiation, favorisent particulièrement la dissémination des bactéries intestinales : d'une part, en affaiblissant la protection immunologique de la barrière intestinale, d'autre part, en déprimant fortement dans le torrent circulatoire la classe d'immunoglobuline la plus adaptée à les combattre (Bazin, 1970b).

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The metabolism of different immunoglobulin classes in irradiated mice

I. CATABOLISM

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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

I. CATABOLISM

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Summary. The catabolism of the different classes of immunoglobulins (Ig) of normal and irradiated mice was studied. A lethal dose of X-rays brought about no significant alterations in the catabolic rates of IgM and IgA, which kept to a time of about 12 hours. Contrariwise, the IgG (IgG₁, IgG_{2a} and IgG_{2b}) displayed a distinctly accelerated catabolism in the irradiated mice, the half-life of these Ig's being reduced by approximately one-half. We propose an interpretation of this phenomenon, based, on one hand, on the great radiosensitivity of the intestinal epithelium and, on the other hand, on the mode of catabolism of IgM and IgA which appears to be different from that of IgG.

INTRODUCTION

It is well established that a drop occurs in the serum concentration of γ -globulins of mice in the days following whole-body irradiation (Grabar, Kashkin and Courcon, 1963; Sassen, Kennes and Maisin, 1966). The different classes of immunoglobulins (Ig): IgM, IgA and IgG, seem to differ with respect to their sensitivities to irradiation (Bazin and Micklem, 1967; Bazin, 1968). This phenomenon may be due to a number of causes. Irradiation is likely to damage the Ig-producing cells, but it is unlikely that irradiation at a dose that can bring about the haemopoietic death of an animal could diminish, at the cell stage, an Ig synthesis already begun. Reuter, Sassen and Kennes (1967b) have shown that mice are still capable of synthesizing γ -globulins 7 days after irradiation at a dose of 700 or 1000 r. Likewise, one of us has shown that mice still synthesize IgM, IgA, IgG₁ and IgG_{2a} between the 24th and 48th hour following a lethal X-ray dose (Bazin, 1968). Lastly, a study of the immunological response of spleen cells of mice immunized against sheep red blood cells has demonstrated that mature cells engaged in the synthesis of specific antibodies are highly radio-resistant (Makinodan, Nettesheim, Morita and Chadwick, 1967). Quite different factors may, however, be operative. Thus Friedberg (1960) found changes in the overall water content of the organism after irradiation. Possible modifications in the distribution of fluid between the intra- and extravascular compartments may be related to this phenomenon (Reuter, Gerber, Kennes and Remy-Defraigne, 1967a). The catabolism of γ -globulins, too, seems subject to modification by irradiation (Reuter *et al.*, 1967b). Hollingsworth (1950), however, has shown that, in rabbits, a dose of 300 r does not alter the rate of decay of transferred antibodies. Furthermore, Perkins and Marcus (1957) found that an irradiation at doses higher than LD₁₀₀/30dH does not reduce the titre of previously produced antibodies. In these two publications there is no mention of the

classes of Ig involved. As a consequence, these various results still leave room for doubt, since the different Ig classes were not studied separately. Considering that the different Igs have very dissimilar metabolisms (Bazin, 1967b), it is possible that the disturbances set up by irradiation may affect the catabolism of each Ig in an individual manner.

In this work we have studied the catabolism of the five classes or sub-classes of mouse Ig (Fahey, Wunderlich and Mishell, 1964a, b).

MATERIALS AND METHODS

Animals

Mice from pure XVII, C3H, C57/BL and BALB/c strains were employed. The XVII animals were used for the catabolism studies and the C3H and BALB/c animals as recipients for mouse myeloma proteins. The C57/BL mice furnished the serum needed for the isolation of normal IgG.

Irradiation

Certain XVII mice were subjected to whole-body irradiation with an X-ray dose of 950 r (225 kV, 12 mA, filtration 0.3 mm Cu and 2 mm Al, cathode-to-target distance 40 cm), which normally produces a 100 per cent animal mortality rate 9–12 days after irradiation.

Nomenclature of immunoglobulins

In continuity with our preceding publication (Bazin, 1966), we here employ the following nomenclature for mouse immunoglobulins, slightly modified from that of Fahey *et al.* (1964a, b): IgM (or β_{2M}), IgA (or β_{2A}), IgG₁ (or 7S γ_1), IgG_{2a} (or 7S γ_{2a}), IgG_{2b} (or 7S γ_{2b}). Synonyms for these names have been listed elsewhere (Bazin, 1967b).

Titration of immunoglobulins

Control mice were bled at the end of each experiment. Each Ig was titrated by the method of Mancini, Carbonara and Heremans (1965), with specific antisera described in an earlier publication (Bazin, 1966). The only modification consisted in the use of pure IgM, and myeloma proteins for the IgA, IgG₁, IgG_{2a} and IgG_{2b}. Purified solutions of each Ig were assayed by the biuret method (Gornall, Bardawill and David, 1949) against a solution of bovine serum albumin titrated by Kjeldahl, and were used as references. In the case of the IgG classes, analyses of the purified solutions always revealed some contaminants belonging to other Ig classes or sub-classes. Because of this, the values obtained were slightly overestimated. The IgG_{2b} was not assayed separately.

Preparation of Ig labelled with iodine-131

(a) *Extraction of Ig.* The IgM was isolated from a mixture of sera from normal mice of XVII or C3H strains by a method already described (Bazin, Savin and Micklem, 1968).

The three IgA preparations used in this study were obtained from serum of BALB/c mice with E III 8 myeloma, given to us by Dr G. Lespinats and Dr G. Hermann. Fifteen millilitres of this serum were applied to a Sephadex G-200 (Pharmacia) column (5 × 100 cm, Tris-HCl buffer, pH 8.0, 0.1 M + 1 M NaCl) and, using the Ouchterlony method, we recovered the myeloma concentrate from the eluate, reconcentrated it and passed it a second time over an identical column. The central portion of the myeloma IgA elution peak was then chromatographed on ion-exchange cellulose. For experiments C4 and C5

we used a DEAE-cellulose (DE-52, Whatman) column (2.5×40 cm) equilibrated with a sodium phosphate buffer, pH 5.5, 0.2 M. The protein solution was dialysed against the buffer, applied to the column, and eluted with the same buffer. Next, the peak eluted with a sodium phosphate buffer (pH 4.8, 0.3 M) was precipitated with half-saturated cold ammonium sulphate. For experiment C6 we used a CM-cellulose (CM-52, Whatman) column, and the peak employed was collected with an 0.05M sodium phosphate buffer of pH 6.5, the column having been washed beforehand with an 0.175 M phosphate buffer of pH 5.5. This peak was also precipitated three times with half-saturated ammonium sulphate.

Isolation of normal IgG. Normal IgG was isolated from serum of C57/BL mice immunized by two intraperitoneal injections, 8 days apart, of 600×10^6 killed *Bordetella pertussis* organisms (Institut Pasteur) suspended in 0.10 ml of Propidon (Specia). The mice were bled 15 days after the second injection. Ten millilitres of the pooled sera were applied to a Sephadex G-25 S (Pharmacia) column by a method already described (Bazin *et al.*, 1968). The proteins were eluted with a sodium phosphate buffer (pH 7.4, 0.1 M) with 0.15 M NaCl added. The second portion of the second eluate peak was concentrated against Aquacid (Calbiochem), and applied to a Sephadex G-200 column (100×2.5 cm). The middle of the second elution peak was considered to represent a purified solution of normal IgG.

Isolation of IgG₁. The IgG₁ solutions for experiments C8 and C10 were obtained from serum of BALB/c mice with myeloma VII A2, given to us by Dr G. Lespinats and Dr G. Hermann. Fifteen millilitres of mouse serum were dialysed against 0.0175 M sodium phosphate buffer of pH 8.0, and applied to a DEAE-cellulose (DE-52, Whatman) column equilibrated with the same buffer. The breakthrough fraction collected with the same buffer was discarded. The proteins eluted with a similar buffer of molarity 0.05 M were precipitated three times with cold half-saturated ammonium sulphate, and chromatographed on a Sephadex G-200 (Pharmacia) column (100×2.5 cm) with an 0.1 M Tris-HCl buffer of pH 8.0 containing 1 M NaCl. The central portion of the second peak was taken as a purified solution of IgG₁ after concentration by ultrafiltration. The solution used for experiment C9 was obtained in the same way, but from 10 ml of serum from mice with MOPC/70A myeloma, originating from Dr M. Potter's laboratory. This serum was given to us by Dr J. F. Doré.

Isolation of IgG_{2a}. The purified solutions of IgG_{2a} were prepared by the method of Askonas (1961) from serum of C3H mice with myeloma 5563, given to us by Dr M. Fougereau.

Isolation of IgG_{2b}. The IgG_{2b} was prepared from serum of BALB/c mice with tumour S188, given to us by Dr M. Cohn. Ten millilitres of serum dialysed against an 0.0175 M sodium phosphate buffer of pH 6.5 were applied on a 40×2.4 cm column packed with DEAE-cellulose (DE-52, Whatman) equilibrated with the same buffer. The breakthrough fraction was discarded. The proteins eluted with an 0.035 M phosphate buffer of the same pH were precipitated three times with cold half-saturated ammonium sulphate. After a short dialysis against a Tris-HCl buffer (pH 8.0, 0.1 M, +1 M NaCl) these proteins were applied on a 100×4 cm Sephadex G-200 (Pharmacia) column. The eluting buffer was the same as already described for the dialysis preceding this filtration on gel. The middle fraction of the second peak of this chromatography was considered to be pure IgG_{2b}. The two samples used in experiments C14 and C15 were prepared separately.

(b) *Labelling with iodine-131.* The various Ig preparations were labelled by the McFarlane (1958) method, modified only to the extent of removing the free iodine on a Sephadex G-50 column (30×2 cm), followed by overnight dialysis at $+4^\circ$ against saline.

(c) *Control of purity of proteins and labelling.* All the ^{131}I -labelled Ig preparations were analysed by immunoelectrophoresis or Ouchterlony analysis. The labelling was verified by radioautography of these immunodiffusion plates, at protein concentrations of the different samples varying from 3 to 10 mg/ml, as already described (Bazin, 1966, 1967a). Exposure times of radioautographs varied from 2 to 30 days. The samples were taken either before injection or at the middle or end of the experiments. All the injected Igs underwent checks at least twice during each study.

Experimental procedures

All the animals were kept in cages containing wood shavings which were changed very frequently. The water, freely available, contained 0.45 per cent of NaCl and 0.01 per cent of KI. Food was provided *ad libitum*. The mice received an intravenous injection of 1–5 μCi of a radioactive solution in a volume of 0.2–0.5 ml. The amount of immunoglobulin injected into each mouse was 0.50–0.75, 0.4–0.6 and 1–2 mg of IgM, IgA and IgG, respectively.

The total radioactivities of the experimental animals were measured by means of a scintillation spectrometer. The mice were confined in a Plexiglass tube which greatly restricted their movements, and were placed at a constant distance from the scintillation crystal, so as to obtain satisfactory and reproducible geometry. The radioactivity was determined 30 minutes after the inoculation and daily thereafter. In the other experiments, in order to evaluate the serum radioactivity, samples of about 60 μl of blood were tapped from the orbital sinus (Pettit, 1913), 30 minutes after the injection and thereafter every 3 hours for the IgM and IgA, or every 24 hours for the IgG. The microhaematocrit tube used for the sampling was centrifuged and then 20 μl were placed, by means of a precision micropipette, in a small tube which was hermetically sealed. The sera were kept at -20° and counted together at the end of each experiment, in a crystal well connected to a spectrometer.

Calculations

(a) *Irradiation due to the injection of ^{131}I -labelled Ig.* The maximum limit of the dose absorbed by the organs of the test animals during the first biological half-life period were evaluated as a function of the activity injected with each Ig preparation.

This maximum limit can be expressed as:

$$D_t = D_\infty \left(1 - 0.5 e^{-0.693 \frac{t}{T}} \right),$$

with

$$D_\infty = \frac{A_0 m_0 E G}{100 M} \times \frac{1}{0.693 \left(\frac{1}{T} + \frac{1}{t} \right)},$$

where $A_0 m_0$ is the initial activity;

M is the average weight of the mice, in grams;

E is the average energy (of the β radiation) in ergs;

G is the geometrical factor;

T is the radioactive half-life of iodine-131, in seconds; and

t is the biological half-life of the Ig under consideration, in seconds.

(b) *Rate of catabolism.* The rate of catabolism of each Ig was estimated from its biological half-life ($T_{1/2}$), which was determined graphically from the data from each experiment. The radioactivity values were expressed as percentages of the inoculated dose. They were corrected for physical decrease of the isotope in the cases where the determinations were based on the whole body radioactivity counts. The radioactive half-life of iodine-131 was taken to be 8.075 days.

RESULTS

IgM

It proved difficult to determine the catabolism of this protein by measuring the decay of the overall radioactivity of the normal and irradiated mice inoculated with ^{131}I -labelled IgM. Thirty-six hours after the injection a large part of the radioisotope still present was concentrated in the liver. On the other hand, there was only slight radioactivity left in the serum, as determined by direct counts. Nevertheless, by radioautography of Ouchterlony plates made with this serum, it was demonstrated that this radioactivity did in fact correspond to IgM, but the amounts were very small.

Furthermore, the shoulder in the whole-body radioactivity decay curve for these animals suggested that the direct counting method was probably not correct for proteins with short half-lives. In such a case, indeed, it appears that one cannot disregard the fate of the catabolized molecules and their decay products which, by their own half-lives, significantly contribute to the whole-body radioactivity count.

Hence the results here described were obtained by measurement of the serum radioactivity of the mice inoculated with ^{131}I -labelled IgM.

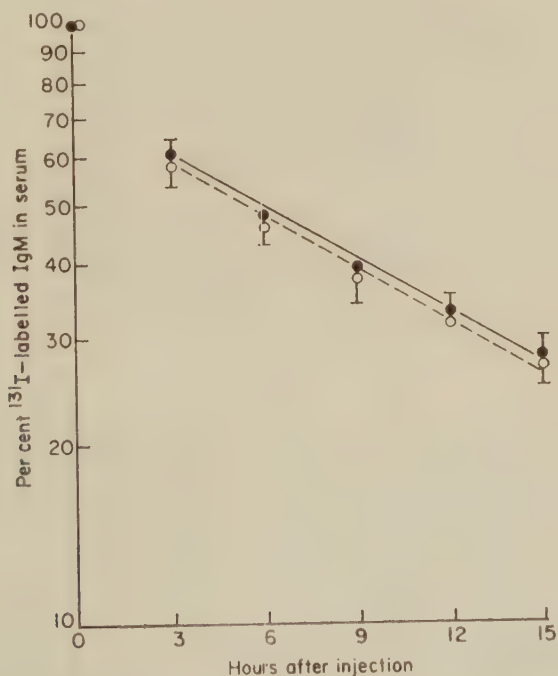


FIG. 1. Catabolism of an ^{131}I -labelled IgM solution in normal (●) and irradiated (○) mice (experiment No. 2). The vertical bars represent the ranges of the data observed.

TABLE 1
HALF LIFE OF ^{131}I -LABELLED IgM IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)			Time between irradiation and ^{131}I -labelled Ig	Half-life of ^{131}I -labelled IgM (hours)
					IgM	IgA	IgG ₁		
C1	200	M	4	0	1.07	0.20	0.34	—	12½
			4	950	—	—	—	-0.5 hours	12
C2	220	M	4	0	2.63	0.19	0.34	—	10½
			4	950	—	—	—	+ 5 days	10
C3	110	F	4	0	0.98	0.09	0.31	—	12
			4	950	—	—	—	+ 5 days	12

TABLE 2
HALF-LIFE OF ^{131}I -LABELLED IgA IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)			Time between irradiation and injection of IgA	Half-life of ^{131}I -labelled IgA (hours)
					IgM	IgA	IgG ₁		
C4	230*	F	4	0	1.28	0.66	0.49	—	13
			4	950	—	—	—	-0.5 hours	13
C5	300	F	4	0	1.46	0.26	1.24	—	11
			4	950	—	—	—	+ 5 days	11
C6	130	M	4	0	2.48	0.18	1.50	—	10
			4	950	—	—	—	+ 5 days	10

* 0.2 ml *Bordetella pertussis* vaccine (6×10^8 organisms) given intraperitoneally at age 60 days and 90 days.

Three different solutions of IgM from normal mice were studied in the XVII mice. The biological half-lives found during these experiments are given in Table 1. Fig. 1 shows the serum radioactivity decay, expressed as a percentage of the inoculated dose, in the normal or irradiated animals in experiment 2. The verification of the degree of purity and the labelling of the IgM sample used in that experiment is illustrated in Fig. 2. As in experiment C2, the controls for experiment C1 showed no other labelled proteins. The protein solution used in experiment C3, however, showed traces of α -lipoprotein. The half-life of the IgM was found to approximate 12 hours in both the normal animals and those irradiated either 5 days before or 30 minutes after the injection of labelled IgM.

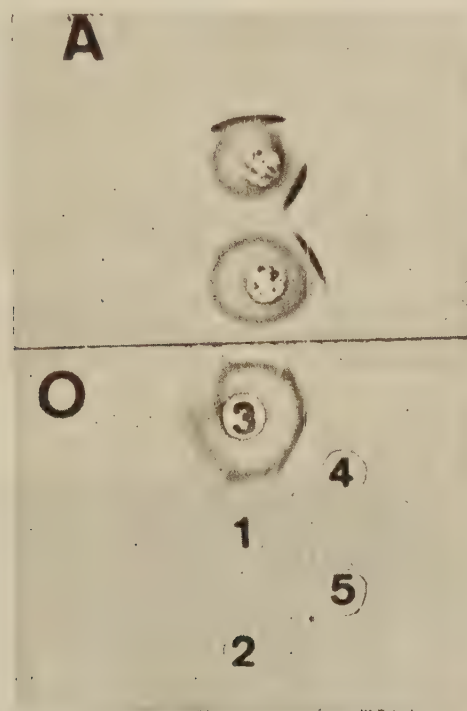


FIG. 2. Ouchterlony test (O), and its radioautography (A), of the ^{131}I -labelled IgM solution used for experiment No. 2. (1) ^{131}I -labelled IgM solution (2 mg/ml); (2) serum taken from an irradiated mouse 3 hours after injection of the labelled solution; (3) rabbit antiserum to whole mouse serum; (4) normal mouse serum; (5) rabbit anti-mouse IgM.

IgA

As with the IgM, the determination of the IgA catabolism was calculated from the decay of the serum radioactivity after intravenous injection of a preparation of ^{131}I -labelled IgA. Three samples of myeloma IgA from plasma-cell tumour E III 8 were studied separately. Table 2 gives the main details of these tests, and Figs. 3 and 4 show the details of experiment C5. In all three cases, the radioautography checks of the protein solutions studied showed that these contained only labelled IgA. In experiment C4, the protein was injected 30 minutes before irradiation, and in experiments C5 and C6, five days after it. No difference in the rate of catabolism could be detected in these three tests. The data show that the IgA half-life is about 12 hours in both the normal and the irradiated mice.

IgG

A mixture of IgG from normal mice, labelled with iodine-131, was injected into XVII mice. Four days later these mice were bled and their serum was inoculated into the four normal and four irradiated (950 r) mice used in experiment C7. This biological screening process enabled the denatured molecules to be eliminated. It certainly also reduced the percentage of IgG_{2b} in the preparation. Fig. 6 shows the immunoelectrophoretic analysis and the radioautography check of this preparation which is a mixture of IgG₁, IgG_{2a} and IgG_{2b}. The half-life of this mixture of normal IgGs in the recipients, as measured from total-body radioactivity (Fig. 5) was 8.5–9 days and 4 days for the normal and irradiated animals, respectively.

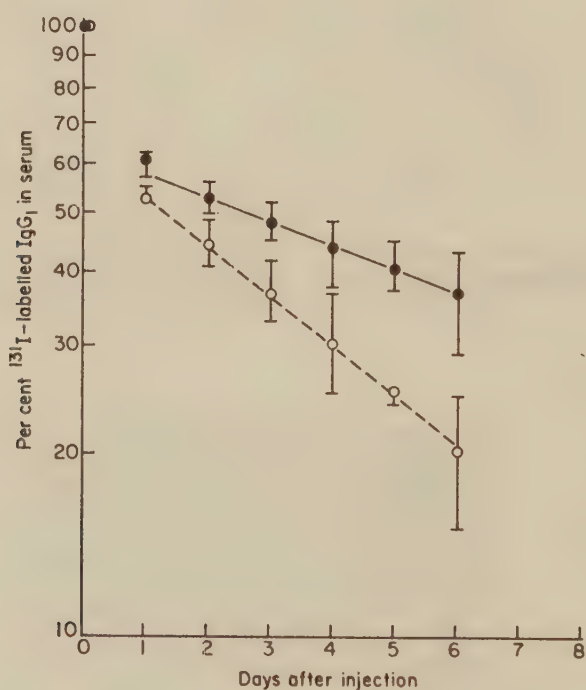


FIG. 7. Catabolism of an ^{131}I -labelled IgG solution in normal (●) and irradiated (○) mice (experiment No. 7). The vertical bars represent the ranges of the data observed.

IgG₁

Two preparations of IgG₁ from the VIIA2 and MOPC/70A myelomas were used in this study (Table 3). Experiments C8 and C9 were effected by whole-body radioactivity counts, and experiment C10 by counts of the serum radioactivity of the inoculated mice. Figs. 7 and 8 show the details of experiment C10. In particular, the ^{131}I -labelled protein appeared to be practically pure, as in the checks effected for experiments C8 and C9. The two first experiments indicated a half-life of about 10 days and 6 days for the normal and irradiated animals, respectively. In the case of the serum radioactivity study, the half-life emerged as 8 and 4 days for the normal and irradiated animals, respectively. This test was performed with the same IgG₁ preparation as was used in experiment C8.

TABLE 3
HALF-LIFE OF ¹³¹I-LABELLED IgG₁ IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	Method of determination	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)				Half-life of ¹³¹ I-labelled IgG ₁ (days)
						IgM	IgA	IgG ₁	IgG ₂	
C8	120	M	Whole body radioactivity	4	0	1.46	0.18	0.49	10.80	11
				4	950	—	—	—	—	6.5
C9	150	F	Whole body radioactivity	4	0	2.36	0.18	0.37	8.20	10
				4	950	—	—	—	—	6
C10	200	M	Serum radioactivity	4	0	1.34	0.21	0.39	16.00	8
				4	950	—	—	—	—	4

TABLE 4
HALF-LIFE OF ¹³¹I-LABELLED IgG_{2a} IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	Method of determination	No. of mice	Irradiation (r)	Mean serum levels (mg/ml)				Half-life of ¹³¹ I-labelled IgG _{2a} (days)
						IgM	IgA	IgG ₁	IgG _{2a}	
C11	230	M	Whole body radioactivity	3	0	0.84	0.19	0.31	35.00	12
				3	950	—	—	—	—	7
C12	180	F	Serum radioactivity	3	0	1.67	0.56	0.48	11.00	6
				3	950	—	—	—	—	4
C13	130	M	Serum radioactivity	4	0	1.16	0.14	0.49	35.00	7
				4 and 4	950	—	—	—	—	4 and 4.5

IgG_{2a}

In the IgG_{2a} catabolism study three samples of myeloma proteins from plasma-cell tumour 5563 were used. Fig. 10 indicates the degree of purity of the preparation employed in the experiment in C12. Besides IgG_{2a}, the sample contained traces of IgG₁ and probably IgG_{2b}. In no case, however, did we detect in these preparations any trace of impurities belonging to serum proteins other than the three IgG categories. The protein solutions in tests C11 and C12 went through an 8-day 'biological screening' before being inoculated into the experimental mice. The tests concerning IgG_{2a} were carried out with the two techniques employed previously. Test C11 was effected by study of the whole-body radioactivity, and C12 and C13 by counting of the serum radioactivity. The main results of these three experiments are summarized in Table 4. Fig. 9 relates to test C13 in which two

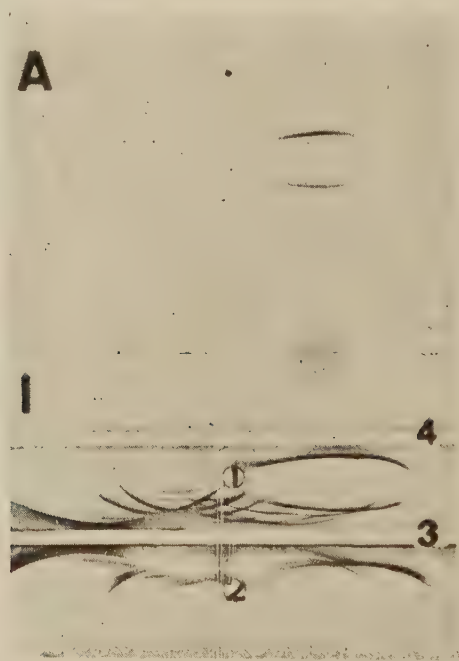


FIG. 8. Immuno-electrophoretic analysis (I) and radioautography (A) of the ¹³¹I-labelled IgG₁ solution used in experiment No. 10. (1) Serum of normal mice under experiment (48 hours after inoculation); (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum; (4) rabbit antiserum to mouse serum IgG.

groups of animals underwent irradiation either 1 hour or 3 days after injection of the labelled IgG_{2a}. The half-life of the preparation used in experiment C11 was found to be 12 days in the controls and 7 days in the irradiated mice. As to experiments C12 and C13, the half-life fell from 6 or 7 days in the controls to about 4 days in the irradiated mice. As IgG_{2a} displays the longest half-life of the different IgG we looked among these groups of animals to find the maximum limit of the irradiation dose due to the ¹³¹I inoculation. The highest reading was found in experiment C11, with about 5 rad.

IgG_{2b}

Two myeloma S188 preparations were used in this work. As can be seen from Fig. 12, the experimental preparation was relatively pure, although slightly contaminated with the

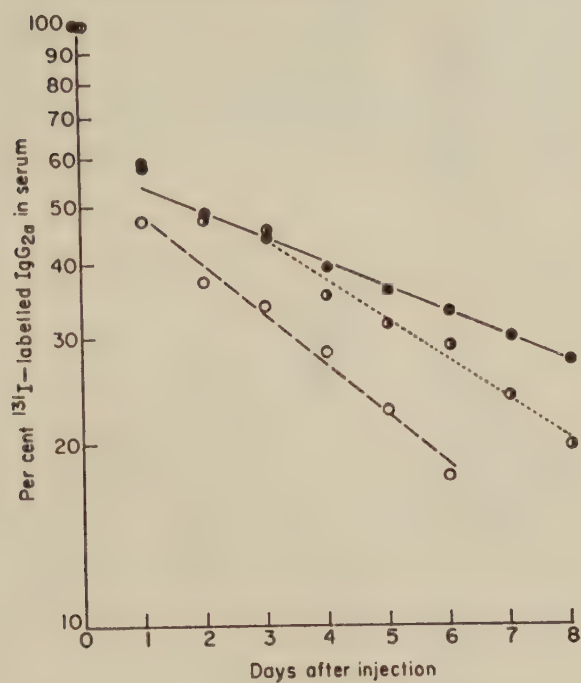


FIG. 9. Catabolism of an ^{131}I -labelled IgG_{2a} solution in normal (●) and irradiated (○) mice at the time of inoculation of the labelled Ig, and 3 days after injection (●).

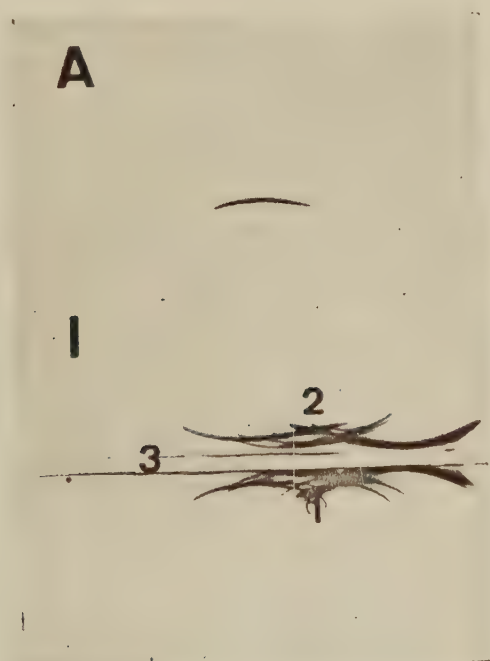


FIG. 10. Immunoelectrophoretic analysis (I) and radioautography (A) of the ^{131}I -labelled IgG_{2a} solution used for experiment No. 13. (1) Mouse serum used, after 'biological screening', to inoculate the experimental mice; (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum.

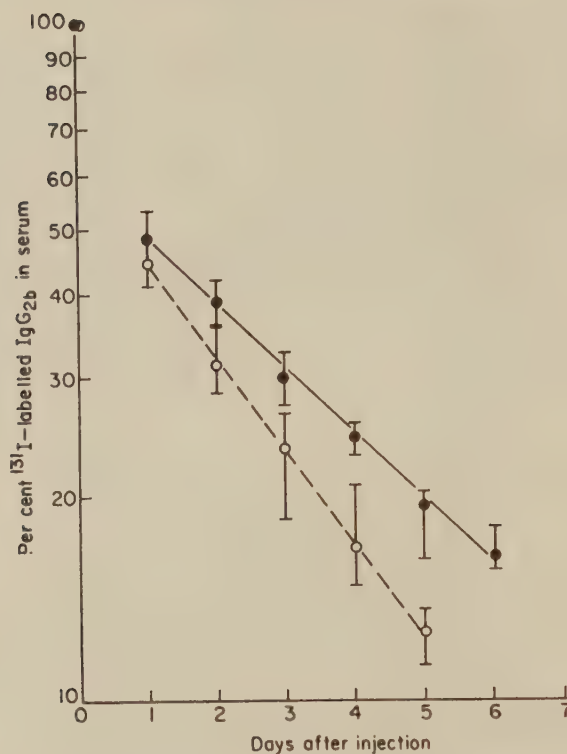


FIG. 11. Catabolism of an ^{131}I -labelled IgG_{2b} solution in normal (●) and irradiated (○) mice. The vertical bars represent the ranges of the experimental data.

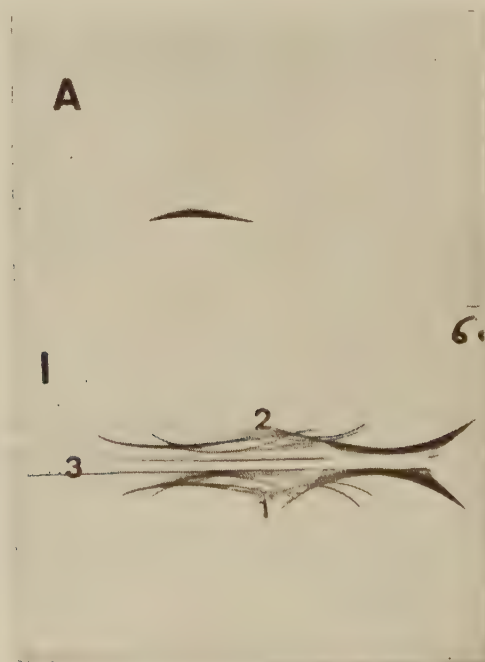


FIG. 12. Immunoelectrophoretic analysis (I) and radioautography (A) of serum of the normal mice in experiment No. 15, taken 3 days after inoculation of the ^{131}I -labelled IgG_{2b} . (1) Serum of the experimental mice; (2) normal mouse serum; (3) rabbit antiserum to whole mouse serum.

other two types of IgG. As the half-life of this IgG₂ sub-class proved to be relatively short, we studied it only by counting the plasma radioactivity after inoculation of the labelled protein solution. Table 5 gives the results of experiments C14 and C15 and Fig. 11 gives the results of experiment C15 in particular. The half-life of the myeloma IgG_{2b} studied was found to range from 3 to 3½ days in the controls and from 2 to 2½ days in the irradiated animals.

DISCUSSION

The experiments described above show that the catabolism of IgM and IgA is only very slightly influenced in the irradiated animals. In no case did we find any difference that could be regarded as significant. On the other hand, the catabolism of IgG₁, IgG_{2a} and IgG_{2b}, as studied by means of myeloma proteins, was found to be distinctly accelerated in the irradiated mice. A mixture of normal IgGs gave a similar result.

The earliest data on Ig catabolism in mice concern the serum 'γ-globulins' without mention of the classes of Ig involved. Dixon, Talmage, Maurer and Deichmiller (1952) found a half-life of 1.9 day, Humphrey and Fahey (1961) 4.6 days and Tee, Watkins and Wang (1965) 4.2 days. Sell (1964) attributed a half-life of 3.2 days to these same proteins, with extremes ranging from 2.5 to 6.2 days. Sell and Fahey (1964) later reported a half-life of 5.4 days with maximum and minimum values of 13.2 and 2.3 days. Lastly Fahey and Sell (1965) published a study of the catabolism of each class of Ig. According to these authors IgM had a half-life of 0.5 day with, in reality, two distinct components corresponding to 0.2 and 0.6 day, respectively. IgA was found to have a half-life of 1.3 day (range: 0.7–1.3 day). Mouse IgG appeared to have a half-life which varied widely with their serum concentrations (Fahey and Robinson, 1963). Depending on whether the experimental animal was exposed to a very weak or very strong microbial environment, or had a paraprotein tumour, these serum concentrations were found to differ greatly. In the conventional animal the half-lives of IgG₁, IgG_{2a} and IgG_{2b} were 4.0, 5.4 and 2.5 days, respectively, with extreme values of 1.8–14, 1.5–11.8 and 0.7–5.5 days. Except in the case of IgM where the values were obtained by the study of haemolysin disappearance (19S), the other results stemmed from measurements of body radioactivity following the injection of ¹³¹I-labelled Ig.

In the present study of Ig catabolism, two methods were employed, namely whole-body radioactivity or serum radioactivity. We found constantly, and more especially in tests C8 and C10 performed with the same protein solution, that the IgG half-life value calculated from the whole-body radioactivity was greater than the value obtained by study of the serum radioactivity. The difference is probably due to the time taken by the body to excrete the free iodine arising from the breakdown of the labelled molecules (Wetterfors, Liljedahl, Plantin and Birke, 1965). Fahey and Sell (1965) advanced this hypothesis in the case of mouse IgA. It is also possible, as Moretti (1967) observed, that in mice some iodine is eliminated through the skin, and this would presumably distort the results. On the other hand, having given the experimental animals potassium iodide in their drinking water, we did not observe any considerable concentration of iodine-131 in their thyroid gland.

TABLE 5
HALF-LIFE OF ¹³¹I-LABELLED IgG_{2b} IN NORMAL OR IRRADIATED MICE

Experiment	Age of mice (days)	Sex	Method of determination	No. of mice	Irradiation (r)	Mean serum Ig levels (mg/ml)				Half-life of ¹³¹ I-labelled IgG _{2b} (days)
						IgM	IgA	IgG ₁	IgG ₂	
C14	210	M	Serum radioactivity	4	0	3.91	0.29	0.51	8.90	3.5
				4	950	—	—	—	—	2.5
C15	100	M	Serum radioactivity	4	0	1.55	0.21	0.43	21.00	3
				4	950	—	—	—	—	2

When studying the influence of X-ray irradiation on the catabolism of Ig, an important point to note is the dose absorbed by the experimental animals which is due to the inoculation of radioactive iodine. Considering only the energy released by the β -rays of the ^{131}I , we calculated a maximum dose of 5 rad in the case of experiment C11. Such a dose, spread over 8 days, does not seem to be capable of significantly disturbing the control animals or the animals irradiated with X-rays.

A problem to be borne in mind is the possibility of denaturation of the injected proteins. Although the magnitude of this factor was not assessed by studies of the excretion of radioactive iodine in the urine, there are reasons to believe that it has not played a significant rôle in our results. Firstly, McFarlane's (1958) method was chosen for the iodination because it has been stated not to denature the proteins (Freeman, 1967). Furthermore, we worked with labelling rates well below 0.5 iodine atom per molecule of IgM or IgA and below one iodine atom per IgG molecule (Freeman, 1967). Whenever possible the injected samples had been submitted to prior 'biological screening' by passage through a primary recipient mouse. Each metabolic experiment was carried out with a separate preparation, except in the case of C8 and C10. This diversity of origin and treatment of the samples employed did not perceptibly affect the reproducibility of the results. Finally, in every case, extrapolation of the straight terminal segment of the plasma decay curve to the vertical axis gave intercepts suggesting extravascular-intravascular distribution ratios compatible with values for IgM, IgA and IgG published in the literature (Schultze and Heremans, 1966). The size of the extravascular compartment would, on the other hand, greatly have been overestimated by this method if large amounts of swiftly catabolized material had been present in the preparations.

It must be noted that the injected amounts of IgM or IgA of each sort were fairly considerable and may have raised the serum level of these proteins. The catabolism of IgA or IgM, however, is independent of their concentration in the blood (Fahey and Sell, 1965). As to the amounts of IgG injected, although relatively high, they are, in our opinion, negligible. One must consider, first, that as regards the IgG_{2a}, its level is high in the serum of our experimental animals. More significantly, the distribution of these proteins between the intra- and extravascular compartments is roughly half-and-half (Schultze and Heremans, 1966), a fact which indicates a very large total pool. Lastly, the findings of Fahey and Robinson (1963) show that a quantity of 1-2 mg given in a single injection should not significantly disturb the catabolism of these proteins.

Slight denaturation due to the isolation procedure might have caused slight and uniform acceleration of the catabolism of the proteins studied. In most cases, however, a denatured preparation will show greatly accelerated initial catabolism, indicating the rapid elimination of grossly damaged molecules, followed by normal catabolism for the remaining intact molecules (Freeman, 1967). In the present study, however, the same half-life was found for ^{131}I -labelled IgG_{2a} which had or had not undergone 'biological screening'.

Another important problem is that of the purity of the normal and myeloma proteins injected. On one occasion the IgM was found to be contaminated with traces of α -lipoprotein. The control tests of the IgA showed it to be pure. The IgG₁, IgG_{2a} and IgG_{2b} solutions, however, were definitely not. These preparations are only mixtures in which one constituent is predominant, owing to the relative proportions of these different Igs in the initial myeloma serum. In no case, however, did we detect any serum constituents other than IgG. The accuracy of immunodiffusion techniques depends on numerous factors,

and the designation 'pure' means little in itself because there are no set standards permitting comparison. On the other hand, radioautography of Ouchterlony or immunoelectrophoresis plates seems to be a far more conclusive test because lines invisible after staining can be revealed by this method.

Each protein sample inoculated was catabolized at a nearly identical rate in all test animals, but appreciable variation was encountered from one sample to the next. For this reason no importance can be attached to comparisons based on samples of different origin. The variations imputable either to the different protein preparations or to the animal groups greatly exceed the experimental errors detected within each test.

In order to try to explain the variations in the catabolism of IgG, a number of factors have to be considered. Firstly one must take into account possible shifts of the fluid distribution ratio between the extra- and intravascular compartments and of the total plasma volume. As already mentioned, changes of this kind do occur in irradiated animals (Friedberg, 1960; Wetterfors *et al.*, 1965). Reuter *et al.* (1967a) considered that the relative increase of the extravascular compartment in relation to the total plasma volume appeared to account for the enhanced catabolism of γ -globulins in irradiated rats. This, however, could not be the cause of the increased IgG catabolism here observed, for this phenomenon occurred both in the whole-body and the serum radioactivity measurements.

Fahey and Robinson (1963) showed that the catabolism of mouse IgGs is linked to the concentration of these proteins in the serum. There seems to be only a single regulating mechanism, dependent on components of the Fc fragment of the molecules. Within certain limits, the rate of catabolism increases when the overall level of these Igs rises and vice-versa. Hence the paradoxical observation of an increased rate of IgG catabolism together with a decreased IgG serum level must signify, not compensation only, but actual overrunning of this regulating mechanism.

Several other, secondary, explanations can be advanced. For instance, it is known that changes in the reticulo-endothelial system may cause acceleration of the catabolism of plasma proteins (Schultze and Heremans, 1966). Hormonal influences on protein catabolism are known to exist and may perhaps have played a rôle in our experiments, at least as regards the adrenocortical hormones (Rothschild, Oratz and Schreiber, 1967). These two explanations are not very satisfactory, however, for they presumably apply to all the serum proteins.

Yet the different immunoglobulins do not seem to be degraded indentially at the various catabolic sites. The fast-catabolizing IgM and IgA seem to be essentially sensitive to an endogenous catabolism process. Conversely, the IgGs, partly protected against that process (Brambell, Hemmings and Morris, 1964), are far more susceptible to intestinal catabolism. There is no doubt as to the reality of the intestinal lesions that follow irradiation (Regaud, Nogier and Lacassagne, 1912). They could significantly increase the IgG catabolism in a selective manner, by increasing the intestinal losses, which constitute the main pathway of degradation for this class of immunoglobulins (Schultze and Heremans, 1966). On the other hand, in the case of the IgM and IgA, the increase of intestinal losses could be considered negligible by comparison with the endogenous catabolism, which would be very little altered. The albumin and IgG seem to be the two kinds of serum proteins most affected by this intestinal catabolism (Schultze and Heremans, 1966). There have been numerous studies on the catabolic sites of albumin. Wetterfors *et al.* (1965) and Lefaire, Cier and Cier (1966) showed that there is a shortening of the albumin half-life in irradiated rabbits. Furthermore, Palmer and Sullivan (1959) demonstrated that the

albumin level could be lowered in rats simply by irradiating the intestine, and Sullivan (1960) as well as Hornsey and Vatistas (1968) showed that polyvinylpyrrolidone (PVP), a substitute for plasma proteins, was massively excreted from the plasma into the lumen of the intestinal tract, after irradiation of the rat and mouse. Hence it is probable that the enhanced IgG catabolism in irradiated mice is indeed due to intestinal lesions: the more so, since in the case of exudative gastroenteropathies it has been shown that the serum IgM level remained remarkably normal, whereas the IgA and IgG levels fell off owing to the intestinal losses (Birke, 1967). This compares well with our results where we found a slight IgM, medium IgG and pronounced IgA decrease in irradiated mice (Bazin and Micklem, 1967; Bazin, 1968). We can, therefore, note that the physiological state of irradiated mice is comparable, as regards the serum Ig levels, to that of an exudative gastroenteropathy in which intestinal protein losses are a confirmed fact. There is still some uncertainty, however, concerning the relative chronology of these intestinal lesions and the increase of catabolism. The return to normal albumin catabolism, in rabbits irradiated with 300 r, occurs between the 9th and the 16th day. At lethal doses the half-life of this protein shows no tendency to normalize before the death of the animals (Leflaure *et al.*, 1966). Our findings with mouse IgG are exactly the same.

It has not been possible, even with the refined methods of quantitation employed, to assign a precise onset to the enhancement of IgG catabolism after irradiation. Using ^{131}I -labelled PVP, however, Hornsey and Vatistas (1968) clearly demonstrated that in mice given a dose of 1000 r, the intestinal losses of the colloid tracer began on the 4th day and remained elevated until about the 9th to 10th day. This type of chronology does not correspond to that of the intestinal lesions, which are apparent already at the 6th hour and have disappeared by the 6th day after irradiation (Duplan, 1966).

It is, however, not certain that the metabolism of the intestinal epithelium is restored to normal at that date (Maisin, 1966). After a lethal irradiation the metabolic disturbances of the intestinal mucous membrane are considerable and independent of its histological aspect.

The present investigation has provided new details on the catabolism of mouse immunoglobulins. It demonstrates that X-ray irradiation very significantly increases the catabolism of the IgG, whilst leaving the IgM and IgA catabolism unchanged. These findings account at least partially for the fact that, in mice, after a lethal irradiation, the serum IgG level shows a greater drop than the IgM level. On the other hand, this work has not thrown any light on the mechanism governing the sharp drop of the serum IgA level which has been observed after irradiation.

Furthermore, it is certain that the different effects of irradiation on the catabolism of the various Ig classes, more especially with regard to the 19S-7S distinction, make for additional difficulty in the study of immunological reactions of irradiated animals. Whether the type of immunity be active, passive or adoptive, the IgM and IgA catabolism will be comparable to that found in the control animals, whereas the IgG catabolism will be increased. In consequence, with one and the same production of haemolysin for instance, the serum concentration of IgM and IgA antibodies will be the same in the irradiated and the control animals, whilst an identical synthesis of precipitating antibodies of IgG type will give a lower serum level in the irradiated animals than in the controls.

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The metabolism of different immunoglobulin classes in irradiated mice

II. ROLE OF THE GUT

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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

II. ROLE OF THE GUT

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Summary. Adult C₃H mice were irradiated by 700 r *in toto* or with the gut shielded. In similar experiments the gut alone was irradiated. The earlier observation of a selective and profound decrease of serum IgA, moderate decrease in IgG₁ and IgG₂, and little change in IgM, was confirmed for the animals irradiated without protection of the gut. Similar effects were found when the gut alone was irradiated, whereas whole-body irradiation with the gut shielded caused only little change in the different serum immunoglobulins. Hence the characteristic responses of the serum immunoglobulins appeared to result from X-ray damage to the intestine. From this and a preceding study it was hypothesized that such damage resulted in moderate losses of IgG from the blood stream into the intestinal lumen, but that the selective and severe depression of serum IgA was mostly due to the escape, through the damaged epithelium, of that portion of the secretion from intestinal plasma cells which normally would have joined the blood stream.

INTRODUCTION

It has been shown (Bazin and Micklem, 1967; Bazin, 1968) that the serum levels of the different immunoglobulin classes in mice respond differently to irradiation by X-rays. On the 9th or 10th day after a lethal dose of X-rays, the concentrations of IgM, IgA and IgG were found to have dropped to 65, 15-20 and 35-50 per cent, respectively, of their initial value.

Tracer studies undertaken to elucidate these phenomena (Bazin and Malet, 1969) have shown that the fractional catabolic rates of IgM and IgA remained unchanged after irradiation, whereas the fractional catabolic rates of the three subclasses of IgG (IgG₁, IgG_{2a} and IgG_{2b}) were significantly increased.

The digestive tract has been described as an important site of catabolism of albumin (Birke, Liljedahl, Olhagen, Plantin and Ahlinder, 1963) as well as of immunoglobulins (Andersen, Glenert and Wallerik, 1963). On the other hand, the gastro-intestinal mucosae of the mouse are known to harbour a vast population of plasma cells containing IgA

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(Crabbé, Bazin, Eyssen and Heremans, 1968), and are noteworthy for their exquisite radiosensitivity (Regaud, Nogier and Lacassagne, 1912).

It therefore seemed warranted to undertake an investigation on the possible role of the intestinal tract in the metabolic changes of immunoglobulins in irradiated mice. This was done by different procedures in which either the gut was shielded while the animal was being irradiated, or the gut alone was exposed to X-rays to the exclusion of the rest of the animal.

MATERIALS AND METHODS

Animals

All animals were 2–3-months-old male C3H mice from a stock bred at the University of Louvain.

Experimental techniques

Surgical preparation of the animals

The mice were anaesthetized by a 1 ml intraperitoneal dose of Rectanol® (Laboratoire Robert & Carrière, Paris), diluted to 1 per cent with saline.

The abdominal skin was incised along the midline over a length of about 3 cm, and a 2 cm long incision was made through the muscular and peritoneal wall. The small intestine, caecum and part of the colon were exteriorized. The animals were constrained lying on their side, with the exposed gut on a pad of gauze wetted with saline.

Irradiation

(a) *Irradiation restricted to the gut.* For this purpose the local irradiation technique described by Maldague (1966) was adapted to the gut. The cylindrical localizer of the X-ray tube (General Electric 'Maxitron', 250 kV, 1 Al+0.25 Cu filtration) was lowered so as to make contact with the mesenteric pedicle. The spleen and stomach, as well as the rectum and part of the colon, remained in the peritoneal cavity and were not exposed to irradiation. The mesenteric lymph node mass, however, was included among the irradiated tissues. The dose of X-rays given in this kind of experiment was 750 r.

(b) *Shielding of the gut in whole-body irradiated animals.* The exteriorized gut was shielded by introducing it into a specially devised lead chamber together with the pad of saline-wetted gauze by which it was protected. This chamber consisted of a flat square box with 2 cm thick walls and removable lid. On one side a 20×2 mm rectangular opening was left between bottom and lid, to allow passage of the mesenteric pedicle. This technique provided effective shielding of the greater part of the gut and the mesenteric lymph nodes. The duodenal loop, the rectum and the terminal portion of the large bowel were however irradiated. The dose of X-rays in this type of experiment was 700 r.

Post-operative care

After irradiation the abdominal wall was sutured at two levels: the muscular and peritoneal layer by means of No. 00 catgut and the skin by means of surgical silk.

The animals were housed together and allowed free access to water and food.

Titration of immunoglobulins

Blood samples were obtained from the retro-orbital sinus just prior to irradiation and on day 5 thereafter. The concentrations of IgM, IgA, IgG₁ and IgG_{2a} (hereafter called IgG₂) in the serum were measured by single radial immunodiffusion (Mancini, Carbonara and Heremans, 1965), using specific antisera as described in a preceding publication (Bazin, 1966).

RESULTS

IRRADIATION RESTRICTED TO THE GUT

Fourteen mice were anaesthetized and their intestinal tract exposed in the manner described. Of these, seven received a dose of 750 r on the exteriorized viscera, whereas the rest served as controls. All animals survived the experiment.

The results of this experiment are given in Tables 1 and 2. The statistical significance of the differences between irradiated and control animals is also indicated in Table 1. The manipulation exposing the intestinal tract, without irradiation, hardly affected the levels of the different immunoglobulins except for a slight fall in IgA. Irradiation of the exposed gut in contrast produced dramatic falls in three of the four immunoglobulins, the exception being IgM. The great decrease in the serum level of IgA was by far the most striking finding in this series of animals.

TABLE 1
SERUM IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING LOCAL IRRADIATION OF THE GUT

	IgM	IgA	IgG ₁	IgG ₂
Gut exposed, no irradiation	92 ± 6	82 ± 11	102 ± 7	110 ± 12
Gut exposed and irradiated	81 ± 5	33 ± 6	63 ± 6	50 ± 3
<i>P</i>	Not significant	0.01	0.01	0.01

The figures are averages (± 1 SD) from seven animals and represent the mean concentrations of the different immunoglobulins on day 5, expressed as percentages of the corresponding values determined in the same animals on the day before the experiment.

TABLE 2
DECREASE IN IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING LOCAL IRRADIATION OF THE GUT

	IgM	IgA	IgG ₁	IgG ₂
Gut exposed, no irradiation (<i>n</i> = 7)				
Initial level	1.34 ± 0.12	1.61 ± 0.34	0.88 ± 0.19	2.74 ± 0.60
Decrease at day 5	-0.12 ± 0.7	-0.35 ± 0.20	-0.01 ± 0.07	-0.25 ± 0.35
Gut exposed and irradiated (<i>n</i> = 7)				
Initial level	1.38 ± 0.10	2.04 ± 0.28	1.30 ± 0.24	2.99 ± 0.38
Decrease at day 5	-0.27 ± 0.07	-1.36 ± 0.20	-0.47 ± 0.09	-1.53 ± 0.22

The figures are averages (± 1 SD) from seven irradiated animals and seven non-irradiated controls, and are expressed in arbitrary units. One unit of each immunoglobulin is the concentration of that protein in a pool of normal mouse sera.

TOTAL BODY IRRADIATION WITH THE GUT SHIELDED

Two types of controls were used in this experiment. The first group were animals which received no irradiation, but whose gut was exposed for the same time as applied to the experimental animals. The other controls were mice which received the same dose of X-rays as the experimental animals, but with exposure of the gut. The dose of X-rays in the experimental animals and the second type of control was 700 r. Of the twenty-five animals employed, three died (one non-irradiated mouse and two non-shielded irradiated mice), on the 2nd day after the experiment.

TABLE 3

SERUM IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING WHOLE-BODY IRRADIATION WITH THE GUT SHIELDED

	IgM	IgA	IgG ₁	IgG ₂
No irradiation, gut exposed (<i>n</i> = 7)	94 ± 6	67 ± 8	103 ± 14	93 ± 6
Whole-body irradiation; gut exposed, not shielded (<i>n</i> = 7)	63 ± 2	12 ± 2	66 ± 7	67 ± 3
Whole-body irradiation; gut exposed, shielded (<i>n</i> = 8)	84 ± 6	71 ± 7	84 ± 9	88 ± 4

The figures are averages (± 1 SD) from the numbers of animals indicated in parentheses, and represent the mean concentrations of the different immunoglobulins on day 5, expressed as percentages of the corresponding values determined in the same animals on the day before the experiment.

TABLE 4

DECREASE IN IMMUNOGLOBULIN LEVELS IN MICE AT DAY 5 FOLLOWING WHOLE-BODY IRRADIATION WITH THE GUT SHIELDED

	IgM	IgA	IgG ₁	IgG ₂
No irradiation, gut exposed (<i>n</i> = 7)				
Initial level	1.38 ± 0.11	1.89 ± 0.41	0.48 ± 0.06	1.35 ± 0.07
Decrease at day 5	-0.06 ± 0.08	-0.77 ± 0.31	-0.06 ± 0.04	-0.09 ± 0.07
Whole-body irradiation, gut exposed, not shielded (<i>n</i> = 7)				
Initial level	1.32 ± 0.07	1.65 ± 0.28	0.59 ± 0.14	1.35 ± 0.13
Decrease at day 5	-0.48 ± 0.02	-1.47 ± 0.27	-0.21 ± 0.08	-0.46 ± 0.07
Whole-body irradiation, gut exposed, shielded (<i>n</i> = 8)				
Initial level	1.29 ± 0.06	1.40 ± 0.23	0.60 ± 0.11	1.32 ± 0.05
Decrease at day 5	-0.19 ± 0.07	-0.45 ± 0.14	-0.12 ± 0.05	-0.15 ± 0.06

The figures are averages (± 1 SD) from the indicated number of experimental animals and the two types of controls, and are expressed in arbitrary units. One unit of each immunoglobulin is the concentration of the protein in a pool of normal mouse sera.

The results from this series are indicated in Tables 3 and 4. The findings in the non-irradiated series with the gut exposed were rather similar to those in the same type of control from the preceding series, except that the fall in IgA levels was somewhat more pronounced. Irradiation with exposure but shielding of the gut produced results which did not statistically differ from the findings in non-irradiated controls. The effect merely amounted to a moderate decrease in all immunoglobulins. The animals that received total body irradiation, without any shielding but with exposure of the gut, showed clearly decreased levels of IgM, IgG₁ and IgG₂, and a particularly impressive fall in the concentration of IgA.

DISCUSSION

It had previously been demonstrated (Bazin and Micklem, 1967; Bazin, 1968) that whole-body irradiation in mice is followed by a profound and selective decrease of the IgA level in the serum, and by similar changes of much smaller magnitude in the other serum immunoglobulins, least of all in IgM. These findings were confirmed in the totally irradiated unshielded animals used as controls in the second experiment here described.

The present investigation clearly indicates that the dramatic effect of total body irradiation on the concentration of serum IgA is essentially due to the radiation damage received by the gut. The fall in serum IgA was prevented by shielding the intestine while irradiation of the gut alone was sufficient to produce the typical decrease in serum IgA. These effects were significantly greater than the moderate decline in serum IgA levels caused by the mere extraperitoneal exposure of the gut in control animals.

The levels of IgG in the serum were more influenced by local irradiation of the gut and by total irradiation than by irradiation with the gut shielded. As already reported (Bazin, 1968), the serum level of IgG was clearly but not considerably decreased on day 5 after whole body irradiation. This could also be related to X-ray damage suffered by the intestine, since the effect was obtained with the gut alone irradiated but not in those irradiated *in toto* with the gut shielded. In the latter animals both IgM and IgG showed the same moderate decrease. The protective effect of shielding the intestine was, however, not so clear cut as in the case of IgA.

It must be emphasized that the technique employed did not allow irradiation of the intestinal tract over its entire length, and that the mesenteric lymph node was included among the tissues exposed to X-ray damage. On the other hand, in the experiments where the gut was shielded, such protection was extended to the mesenteric lymph node, and it was impossible to keep the distal and proximal extremities of the intestinal tract from being irradiated. It is possible, therefore, that with a better technique the differences between the results of the two types of experiment might become even more striking than here observed. One may also surmise that the shielded parts of the body suffered slight damage by back-scattering from the irradiation of other tissues. It must also be taken into account that blood was sampled only on day 5 after the experiment. This delay was chosen on the basis of previous findings which had indicated that the greatest differences between the different Ig classes would be observed at that time, though it did not coincide with the moment of maximal depression of the serum IgM and IgG levels.

The serum immunoglobulins clearly fell into three classes so far as the effects of irradiation were concerned. IgM was hardly affected and, as shown here, the gut was not implicated in whatever decline in IgM may be observed. This is not surprising if one recalls that the intestine is only a very minor site of IgM production, as judged from the scarcity of IgM plasma cells in this tissue (Crabbé *et al.*, 1968). It has been shown elsewhere (Bazin and Malet, 1969) that the biological survival of ^{131}I -labelled IgM is not shortened in irradiated mice, indicating that no important intestinal losses of this immunoglobulin occur through the damaged mucosa. The small decrease in IgM serum levels observed at day 5 may be due to radiation damage to the cells producing this immunoglobulin. Doses of the order of 700–750 r have been shown not to inhibit to any great extent those cells which are already secreting antibodies, though the renewal of this cell population is more seriously affected (Kennedy, Til, Siminovitch and McCulloch, 1965; Makinodan, Nettesheim, Morita and Chadwick, 1967).

IgG₁ and IgG₂ in irradiated mice were present at decreased concentrations. The experiments here described indicate that radiation damage to the gut was the major factor involved in this change. It has been shown in a previous study (Bazin and Malet, 1969) that these two immunoglobulins are abnormally rapidly removed from the blood stream after *in toto* irradiation. Taken together, all data suggest that the fall in serum IgG level after irradiation is to a large extent due to abnormal losses through the damaged intestinal mucosa. In this respect, IgG resembles albumin, which is also lost in great amounts during the exudative gastroenteropathy following irradiation (Palmer and Sullivan, 1959). Increased vascular permeability is known to prevail in the irradiated intestinal mucosa (Hornsey and Vatisstas, 1968). However, diminished synthesis may also play a role in the fall of IgG₁ and IgG₂ serum levels. This is suggested by their moderate decrease in control animals having their gut shielded during whole body irradiation (Tables 3 and 4).

IgA is a protein whose concentration in the serum undergoes a very early and pronounced fall after whole-body irradiation (Bazin, 1968). The present experiments clearly indicate that the metabolic lesion responsible for this effect is localized in the gut. Several interpretations may be considered.

Firstly, the intestinal mucosa of the normal adult mouse possesses an extremely dense population of plasma cells containing IgA (Crabbé *et al.*, 1968), and has been shown to synthesize IgA very actively *in vitro* almost to the exclusion of other immunoglobulins (Mandel and Asofsky, 1968). Although much of this IgA is presumably excreted into the lumen of the gut, there is good reason to believe that part of it joins the blood stream to contribute to the pool of circulating IgA. This, at any rate, is what happens in the dog, whose mesenteric lymph (which drains the *lamina propria* of the gut) has been reported to contain IgA in great excess over the amount reaching the mucosa by way of the arterial blood supply (Vaerman and Heremans, 1970). Turnover studies with [¹³¹I]IgA in mice have shown that the half-life of this protein is not significantly shortened after irradiation (Bazin and Malet, 1969). Although these investigations were carried out with a myeloma protein, and need confirmation with regard to normal serum IgA, they seem to indicate that the observed fall in concentration is due to a shutdown of the supply of IgA to the plasma rather than to losses from the blood stream. Escape of IgA from the plasma into the lumen of the damaged gut would at any rate not exceed in proportion that of IgG and hence should not be able to produce the precipitous selective fall in serum IgA which is observed. Hence, one may wonder whether the major metabolic lesion affecting IgA is not simply a suppression of its synthesis by the intestinal plasma cells. This idea is supported by reports on the damaging effect of irradiation on the intestinal plasma cells of the guinea-pig (Ansari, Eder and Nägele, 1962) and the mouse (Deby and Malkany, 1968). Again it may also be relevant to note that X-rays impair the capacity of immunologically competent cells to proliferate (Makinodan *et al.*, 1967).

However, this hypothesis would imply that cells synthesizing IgA—or at least those among them that are localized in the intestinal mucosa—are considerably more vulnerable to X-ray damage than are other varieties of immunoglobulin-producing cells. Such an *ad hoc* postulate lacks experimental support. Rather, immunofluorescence studies on the irradiated mouse gut do not seem to indicate any important decrease in the counts of IgA-type plasma cells (in preparation). Locating the selective sensitivity at the level of the lymphoid precursor cells is equally unsatisfactory, the more since the different types of haematopoietic stem cells are known to be of similar radiosensitivity (Silini, Pons and Pozzi, 1968). One may still argue that the location of IgA-producing cells in a site so

exquisitely radiosensitive as the gut may adversely affect their metabolic functions. However, biosynthesis of IgA in the mouse has been found to persist at the 24th hour following a total body dose (950 r), higher than that employed in the present study (700 r) (Bazin, 1968).

The alternative hypothesis is that the rate of synthesis of IgA by the irradiated intestinal plasma cells is not decreased any more profoundly than that of other immunoglobulins, but that in the irradiated animal the greater part of the IgA secreted in the *lamina propria* escapes into the lumen through the damaged epithelium instead of joining the lymphatics and hence the blood. This interpretation would be consistent with the already mentioned role of the gut as a major supplier of the pool of plasma IgA. Considering the magnitude of the effect observed, one even wonders whether the intestinal mucosa may not be the most important source of IgA in the body. This hypothesis finds support in the knowledge that X-ray doses of the order employed in the present study are able to damage intestinal epithelial cells (Hugon, Maisin and Borgers, 1963; Casarett, 1968). Such lesions have been reported to develop within 6 hours and to persist for 5 or 6 days. Electron-microscopic studies on the intestinal epithelium and immunochemical investigations on the gut content of irradiated mice, now being undertaken at this laboratory, will be reported elsewhere.

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THE METABOLISM OF DIFFERENT IMMUNOGLOBULIN
CLASSES IN IRRADIATED MICE
III. EFFECTS OF SUPRA-LETHAL DOSES OF X-RAYS

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The metabolism of different immunoglobulin classes in irradiated mice

III. Effects of supra-lethal doses of X-rays†

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The serum level of IgM, IgA, IgG₁ and IgG₂ was studied in mice exposed to 1000-5000 R of x-rays. The serum concentration of all classes was found to decrease during the first 72 hours after irradiation. At 72 hours after 2000-5000 R, the percentage of the initial value ranged from 53 to 70 for IgM, from 24 to 44 for IgA, from 63 to 70 for IgG₁, and from 55 to 71 for IgG₂. If several mechanisms may account for the decrease of each Ig class, the more pronounced fall of the serum level of IgA can clearly be related to the radiation induced lesions of the intestinal mucosa.

1. Introduction

Ionizing radiations are known to affect the serum level of immunoglobulins (Ig) (Grabar, Kashkin and Courcon 1963, Sassen, Kennes and Maisin 1966, Bazin and Micklem 1967). Höhne, Künkel and Anger (1955) exposed rats to a supra-lethal dose of 3000 R and found a decrease of serum gamma globulins as determined by zonal electrophoresis. Similar findings were reported by Winkler and Paschke (1956). These studies did not take into account the heterogeneity of Ig and possible specific effects of radiation on the metabolism of each Ig class. As a matter of fact, it was shown in the mouse that the serum levels of Ig classes, namely IgM, IgA and IgG, were differently affected by sub-lethal or lethal doses of x-rays (Bazin and Micklem 1967, Bazin 1968). In the lethal range, the gut radiosensitivity plays a major role in the decrease of the serum levels of IgG and IgA (Bazin, Maldague and Heremans 1970). It seemed interesting to study the effects of supra-lethal doses of radiation, which cause acute intestinal death through severe epithelial lesions, on the serum level of each Ig class. This was investigated in mice exposed to x-ray doses ranging from 1000 to 5000 R.

2. Methods

2.1. Mice

Animals were 6-month-old female (C57B1♀ × C3H♂)F₁ hybrid mice caged in groups of six and allowed free access to pellets of standard food and chlorinated water. Mice of each cage were randomized with regard to the radiation treatment and the time of sacrifice.

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2.2. Irradiation

Mice were given a single whole-body x-ray dose of 1000, 1500, 2000, 3000, 4000 or 5000 R (mid-line air dose) in a perforated Plexiglas container. Irradiation factors: 260 kV; 14 mA; 1 mm Cu + 1 mm Al; h.v.l. 2 mm Cu; anticathode-object distance, 60 cm; dose-rate, 66 R/min.

2.3. Serology

Each mouse was bled 12 hours before and 1, 2, or 3 days after irradiation. At the first bleeding, 0.05 ml. of blood was withdrawn from the orbital sinus. The second bleeding was performed by decapitation. Individual sera were collected and stored at -25°C . Titration of the serum concentration of each Ig class was carried out on each sample by the technique of Mancini, Carbonara and Heremans (1965), using rabbit anti-mouse monospecific antisera and mouse Ig standards prepared as previously described (Bazin and Malet 1969).

Radiation dose (R)	Hours before irradiation 12	Hours after irradiation		
		24	48	72
0	3.30 $\pm$ 0.25 2.86 $\pm$ 0.42 3.82 $\pm$ 0.31	3.18 $\pm$ 0.22	2.82 $\pm$ 0.45	4.00 $\pm$ 0.33
1000	4.65 $\pm$ 0.44 5.15 $\pm$ 0.43 4.10 $\pm$ 0.36	3.90 $\pm$ 0.34	4.04 $\pm$ 0.37	3.60 $\pm$ 0.35
1500	4.44 $\pm$ 0.49 4.59 $\pm$ 0.39 4.10 $\pm$ 0.28	3.95 $\pm$ 0.52	3.32 $\pm$ 0.11	2.73 $\pm$ 0.15
2000	4.85 $\pm$ 0.45 4.62 $\pm$ 0.28 5.06 $\pm$ 0.85	3.81 $\pm$ 0.28	3.29 $\pm$ 0.22	3.13 $\pm$ 0.47
3000	4.79 $\pm$ 0.65 4.38 $\pm$ 0.36 3.46 $\pm$ 0.59	4.02 $\pm$ 0.38	3.12 $\pm$ 0.27	2.42 $\pm$ 0.13
4000	5.05 $\pm$ 0.78 4.33 $\pm$ 0.21 4.58 $\pm$ 0.18	3.97 $\pm$ 0.75	3.65 $\pm$ 0.56	2.44 $\pm$ 0.11
5000	4.31 $\pm$ 0.36 4.04 $\pm$ 0.22 4.51 $\pm$ 0.18	3.17 $\pm$ 0.23	3.03 $\pm$ 0.24	2.48 $\pm$ 0.10

Table 1. Serum concentration of IgM in irradiated and control animals†.

† Values given as mg/ml. $\pm$ one standard error represent the means of six mice.

Immunoglobulin classes were identified as IgM, IgA, IgG₁ and IgG₂, according to the nomenclature previously used (Bazin 1967). IgG₂ refers to IgG_{2a} only, owing to the difficulty of detecting IgG_{2b}.

3. Results

In the present study normal serum levels of the Ig classes were determined on 126 mice. The following values represent the mean (mg/ml.) $\pm$ one standard error: IgM, 4.33 ± 0.10 ; IgA, 0.53 ± 0.02 ; IgG₁, 1.42 ± 0.04 ; IgG₂, 12.9 ± 0.40 . No mortality was observed during the time course of the experiment. Table 1 shows the actual titration data for IgM. The decrease of serum level of IgM as a function of time after irradiation was apparent and similar at all radiation doses. The decline of IgA, as shown in table 2, was much more pronounced as soon as 24 hours after irradiation, especially after doses higher than 1000 R.

Radiation dose (R)	Hours before irradiation 12	Hours after irradiation		
		24	48	72
0	0.40 ± 0.05 0.32 ± 0.05 0.36 ± 0.04	0.36 ± 0.03	0.30 ± 0.05	0.42 ± 0.06
1000	0.60 ± 0.13 0.51 ± 0.06 0.59 ± 0.03	0.62 ± 0.25	0.28 ± 0.01	0.35 ± 0.02
1500	0.59 ± 0.15 0.44 ± 0.06 0.56 ± 0.05	0.32 ± 0.07	0.24 ± 0.04	0.29 ± 0.01
2000	0.65 ± 0.14 0.52 ± 0.05 0.55 ± 0.05	0.38 ± 0.05	0.21 ± 0.03	0.24 ± 0.01
3000	0.68 ± 0.08 0.56 ± 0.11 0.54 ± 0.03	0.34 ± 0.03	0.22 ± 0.01	0.18 ± 0.02
4000	0.51 ± 0.07 0.45 ± 0.04 0.62 ± 0.09	0.30 ± 0.03	0.20 ± 0.01	0.15 ± 0.02
5000	0.57 ± 0.08 0.42 ± 0.03 0.59 ± 0.07	0.27 ± 0.01	0.20 ± 0.02	0.18 ± 0.01

Table 2. Serum concentration of IgA in irradiated and control animals†.

† Values given as mg/ml. $\pm$ one standard error represent the means of six mice.

Tables 3 and 4 refer to IgG₁ and IgG₂, respectively. The concentration of these two immunoglobulins diminished similarly after irradiation. Their decrease was comparable to that of IgM. The relative radiosensitivity of the serum level of the four Ig classes is illustrated in the figure, which represents mean percentages of the initial values for each group of animals. The remarkable radiosensitivity of the serum level of IgA was further confirmed in a subsequent experiment, in which mice were bled 12 hours before and sacrificed 24 hours after 2000 R. The titration data from 11 irradiated and 12 control

Radiation dose (R)	Hours before irradiation 12	Hours after irradiation		
		24	48	72
0	5.30 ± 0.57 4.52 ± 0.28 6.64 ± 0.66	4.99 ± 0.36	3.90 ± 0.36	5.56 ± 0.55
1000	12.53 ± 1.22 12.29 ± 0.68 18.83 ± 1.49	10.99 ± 0.95	10.48 ± 0.44	15.53 ± 1.37
1500	13.40 ± 0.61 13.05 ± 0.28 15.84 ± 0.41	11.52 ± 0.74	10.88 ± 0.43	12.32 ± 0.58
2000	12.89 ± 0.94 13.06 ± 0.70 20.99 ± 2.56	10.62 ± 0.62	9.83 ± 0.45	11.58 ± 0.91
3000	14.57 ± 1.19 13.61 ± 1.62 12.40 ± 1.28	11.28 ± 1.24	8.81 ± 1.35	8.19 ± 0.37
4000	13.13 ± 1.10 13.61 ± 0.86 13.55 ± 0.91	11.09 ± 1.03	8.70 ± 1.10	9.68 ± 1.18
5000	16.78 ± 1.70 11.06 ± 0.65 13.65 ± 0.74	11.95 ± 1.58	9.79 ± 1.00	9.23 ± 0.31

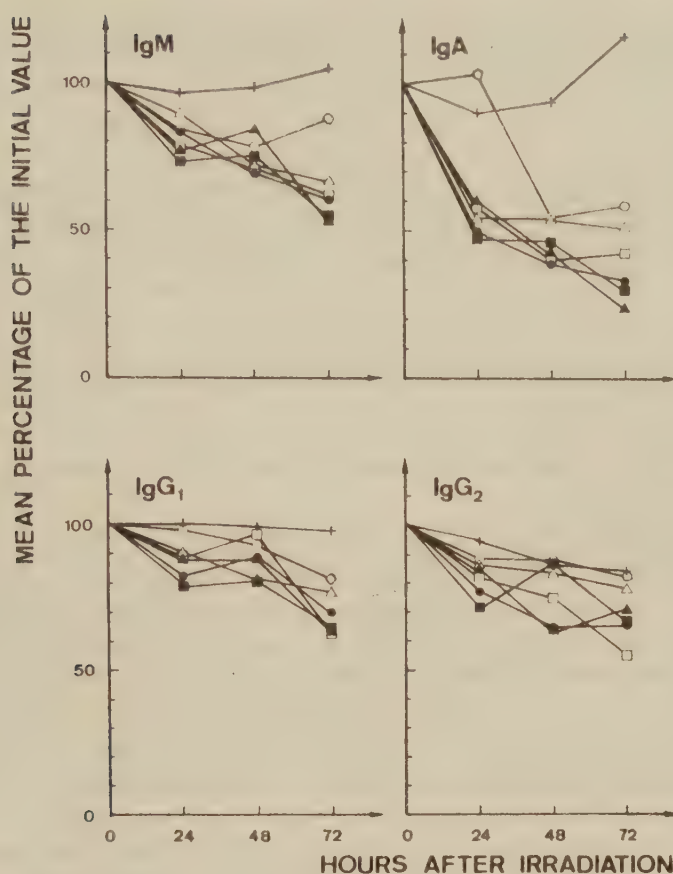
Table 4. Serum concentration of IgG₂ in irradiated and control animals†.

† Values given as mg/ml. ± one standard error represent the means of six mice.

Radiation dose (R)	Hours before irradiation 12	Hours after irradiation		
		24	48	72
0	1.01 ± 0.08 0.97 ± 0.14 0.93 ± 0.09	1.01 ± 0.07	0.96 ± 0.09	0.91 ± 0.08
1000	1.17 ± 0.07 1.16 ± 0.07 2.03 ± 0.16	1.15 ± 0.07	1.08 ± 0.07	1.65 ± 0.16
1500	1.15 ± 0.09 1.34 ± 0.12 1.66 ± 0.09	1.04 ± 0.06	1.09 ± 0.08	1.28 ± 0.09
2000	1.26 ± 0.17 1.07 ± 0.28 1.79 ± 0.19	1.13 ± 0.12	1.04 ± 0.05	1.12 ± 0.14
3000	1.54 ± 0.11 1.51 ± 0.11 1.70 ± 0.07	1.26 ± 0.10	1.35 ± 0.16	1.19 ± 0.12
4000	1.58 ± 0.05 1.41 ± 0.06 1.48 ± 0.16	1.41 ± 0.12	1.25 ± 0.07	0.93 ± 0.06
5000	1.61 ± 0.23 1.54 ± 0.19 1.74 ± 0.22	1.28 ± 0.15	1.26 ± 0.16	1.14 ± 0.12

Table 3. Serum concentration of IgG₁ in irradiated and control animals†.

† Values given as mg/ml. ± one standard error represent the means of six mice.



Mean percentages of the initial values of Ig serum levels as a function of time after irradiation. Initial values were determined 12 hours before irradiation and taken as 100 per cent. Each point refers to six mice, each of which was tested for the serum concentrations of four Ig classes. +—+, unirradiated; ○—○, 1000 R; △—△, 1500 R; □—□, 2000 R; ●—●, 3000 R; ▲—▲, 4000 R; ■—■, 5000 R.

Ig class	Radiation dose	First bleeding	Second bleeding	<i>P</i>
M	0	3.73 ± 0.26	4.21 ± 0.41	> 0.05
	2000	3.43 ± 0.15	3.06 ± 0.11	> 0.05
A	0	0.52 ± 0.05	0.55 ± 0.04	> 0.05
	2000	0.52 ± 0.06	0.32 ± 0.02	< 0.01
G ₁	0	1.18 ± 0.06	1.22 ± 0.07	> 0.05
	2000	1.13 ± 0.07	1.01 ± 0.07	> 0.05
G ₂	0	9.90 ± 0.54	10.68 ± 0.66	> 0.05
	2000	8.88 ± 0.60	8.28 ± 0.42	> 0.05

Table 5. Serum concentration of four Ig classes in irradiated and control animals†.

† Values given as mg/ml. ± one standard error represent the means of 11 irradiated and 12 control mice. First and second bleeding were performed 12 hours before and 24 hours after irradiation. Controls were bled at the same times as the irradiated animals. *P* refers to the significance level of the difference in Ig mean concentration between the first and second bleeding, as evaluated by the *t* test.

mice are presented in table 5. Under these conditions, the effect of radiation was highly significant only for the IgA class.

4. Discussion

Dehydration is known to occur immediately before gastrointestinal death (Bond 1968). The present study was limited to the first 72 hours after irradiation to avoid gross changes of serum protein concentration resulting from hydric imbalance. The results indicate that after supra-lethal doses of x-rays there was a differential decrease of the serum levels of the Ig classes, as already observed after sub-lethal or lethal doses (Bazin and Micklem 1967, Bazin 1968). However, the more pronounced effect of radiation on the serum level of IgA, as compared to the other Ig classes, was magnified by supra-lethal doses. Yet, within the supra-lethal range, similar results were obtained when doses higher than 2000 R were used.

The decrease of the serum level of IgM was slightly greater than the decreases of IgG₁ and IgG₂, which were found quite comparable. The decline of the serum level of these three Ig classes may have resulted from radiation damage on Ig-secreting cells (Kennedy, Till, Siminovitch and McCulloch 1965, Sado 1969) and their precursors (Makinodan, Nettesheim, Morita and Chadwick 1967). Killing of precursor cells might explain the results owing to the finding that Ig-secreting cells are relatively less sensitive to radiation damage (Vann and Makinodan 1969). However, the rapid decline of the serum levels of Ig is hardly compatible with changes of the turnover of these cells. Previous studies showed that the catabolism of IgM, IgG₁ and IgG₂ was not affected during the first 72 hours after 950 R (Hornsey and Vatistas 1968, Bazin and Malet 1969). That supra-lethal doses of x-rays may increase the catabolism of these Ig classes by abnormal extravascular loss cannot be ruled out. However, the case for this mechanism is not supported by the fact that the decrease of the serum level of IgM was slightly larger than that of both IgG₁ and IgG₂. The much greater molecular weight of IgM would, indeed, suggest a preferential extravascular filtration of the IgG₁ and IgG₂.

The more pronounced decrease of the serum level of IgA shortly after supra-lethal doses of x-rays is compatible with the following mechanisms: (a) increased catabolism by extravascular loss; (b) higher radiosensitivity of IgA-producing cells and their precursors; (c) decreased supply of IgA to the serum by direct intestinal loss. The first mechanism seems unlikely, because of the same arguments as those presented above for IgM. The radiosensitivity of IgA-producing cells and their precursors lacks experimental evidence for the supra-lethal range. Previous studies on the effects of lethal doses of x-rays on these cells do not allow the conclusion that the IgA-producing cells are more radiosensitive than cells producing the other Ig classes. Also a differential radiosensitivity among the precursors of Ig-forming cells seems very unlikely (Bazin *et al.* 1970). The intestinal mucosa of the normal adult mouse possesses an extremely dense population of plasma cells containing IgA (Crabbé, Bazin, Eyssen and Heremans 1968). Although much of the secreted IgA is presumably excreted into the lumen of the gut, there is good reason to believe that part of it joins the blood stream to contribute to the circulating pool of IgA. It has been shown that intestinal lesions produced by lethal doses of x-rays may cause a remarkable fall of the IgA serum level (Bazin *et al.* 1970). The selective

depression of serum IgA, as observed already 24 hours after supra-lethal doses, may well be due to the escape through the damaged epithelium of that portion of the secreted IgA that normally would have joined the blood stream. As a matter of fact discontinuity of the epithelial cells is evident after 1350 R as early as 6 hours following irradiation (Hugon, Maisin and Borgers 1963). Thus, mechanism (c) seems to be very likely. Since the serum level of IgA is probably related to the integrity of the gut epithelium, the fast decrease of this specific class may be a very early indicator of the intestinal lesions produced by supra-lethal doses of x-rays.

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We are grateful to Mr. A. Di Michele for the excellent technical assistance.

Les taux sériques des IgM, IgA, IgG₁ et IgG₂ ont été analysés chez des souris irradiées à des doses de rayons x de 1000 à 5000 R. Pour toutes les classes d'Ig, on observe une baisse de leurs concentrations sériques pendant les 72 premières heures après l'exposition aux radiations ionisantes. En particulier, 72 heures après une irradiation de 2000 à 5000 R, les pourcentages de chaque Ig par rapport à leur valeur initiale se rangeaient entre des valeurs de 53 à 70 pour cent pour les IgM, de 24 à 44 per cent pour les IgA, de 63 à 70 pour cent pour les IgG₁ et de 55 à 71 pour cent pour les IgG₂. Si plusieurs mécanismes peuvent être avancés pour expliquer ces décroissances du taux sérique des diverses classes d'Ig, la chute caractéristique de l'IgA peut nettement être rattachée aux lésions de la muqueuse intestinale, induites par les radiations.

Während der drei ersten Tage nach einer Röntgenbestrahlung mit 1000–5000 R wurde bei Mäusen ein progressiver Abfall des Serumspiegels der vier Immunglobuline (IgM, IgA, IgG₁ und IgG₂) festgestellt.

Nach 72 Stunden betrugen die Konzentrationen im Serum—bezogen auf die Ausgangswerte—53 bis 70 Prozent für das IgM, 24–44 Prozent für das IgA, 63–70 Prozent für das IgG₁, sowie 55–71 Prozent für das IgG₂. Obwohl die Pathogenese dieser Veränderungen nicht eindeutig zu sein braucht, so steht die selektiv verstärkte Senkung des Serum-IgA doch zweifellos mit strahleninduzierten Läsionen der Darmschleimhaut in Zusammenhang.

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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

IV. FATE OF CIRCULATING IgA OF TUMOUR OR TRANSFUSION ORIGIN

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Summary. BALB/c conventional mice carrying plasma cell tumour grafts which secrete IgA do not, in contrast to normal mice, experience any decline in their serum IgA levels following wholebody irradiation by 900 R, provided the tumours themselves are protected from X-ray damage.

Germ-free Swiss mice whose serum IgA levels had been raised to near-normal values by a transfusion with normal mouse serum, did not show any selective decline in the serum IgA concentration following whole-body-exposure to 2000 R, in contrast to conventional adult mice in which this class of immunoglobulin is severely and selectively depressed by a similar treatment. Nor did germ-free transfused mice excrete abnormally high quantities of IgA into their intestinal fluids.

It is concluded that the previously described selective fall in serum IgA levels following irradiation does not occur when the source supplying IgA to the serum pool is protected from the action of X-rays. Conversely, this metabolic disturbance cannot be explained by any specific increase of catabolism or intestinal loss of this serum immunoglobulin.

INTRODUCTION

All four major immunoglobulin classes of the mouse, IgG₁, IgG₂, IgA and IgM, undergo a decrease in their serum levels following exposure of the animal to sublethal (Bazin, 1968), lethal (Bazin and Micklem, 1967) or supralethal (Bazin and Doria, 1970) doses of X-rays, but by far the most marked effect is observed with immunoglobulin IgA. For example, in a typical experiment, on the 9th or 10th day after a lethal X-ray dose (950 R), the concentrations of IgG₁, IgG₂ and IgM were found to have decreased to about 40, 55 and 60 per cent of their respective base line values, whereas that of IgA had fallen to 24 per cent of its initial level (Bazin, 1968).

The origin of this selective disturbance in the IgA metabolism resides in the gut, as shown by experiments in which either the whole animal was irradiated with the gut shielded, or the gut alone was irradiated and the rest of the animal protected against the X-rays (Bazin, Maldague and Heremans, 1970). *A priori*, the specific fall in serum IgA levels following X-ray damage to the gut might be ascribed either to selective (intestinal or hypercatabolic) loss of IgA, or to some selective X-ray induced disturbance in the supply of IgA to the circulating pool of this immunoglobulin.

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The first possibility has been rendered unlikely by the demonstration that the half-life of [^{131}I]IgA was not significantly shortened in irradiated mice (Bazin and Malet, 1969). However, before founding any extensive theory on these data it seemed advisable to gather further proof that the metabolic fate of circulating IgA was not essentially different in irradiated mice as compared to controls.

The present study aimed to demonstrate that IgA is not selectively lost from the circulation following whole body irradiation, provided that the supply of this protein from its source of production to the plasma is not interrupted by the experimental procedure.

In the two types of experiment described hereafter, the circulating IgA was almost entirely of extraintestinal origin, being in one case synthesized by a grafted plasma cell tumour, and in the other case derived from the intravenous administration of normal mouse serum.

MATERIALS AND METHODS

Animals

Germ-free Swiss mice, 3 months old, were purchased from Zentralinstitut für Versuchstierzucht of Hannover, W. Germany, and were random bred in Trexler type isolators. They were removed from their isolators just prior to the irradiation procedure and were thereafter kept as described for the 'conventional' group of animals.

'Conventional' (as opposed to germ-free) animals were 3-months old male BALB/c mice which were caged 4-6 together, with free access to standard pellet food and chlorinated drinking water throughout the whole experimental period.

Plasma cell tumours

The tumour used for these studies was the IgA-producing transplantable plasma cell tumour S-121, which was kindly provided by Dr Melvin Cohn. Transplantations were carried out by the subcutaneous route. A dorsal site was chosen so as to allow efficient shielding from X-ray irradiation.

Irradiation procedures

All BALB/c animals, with or without grafted tumours, were maintained under Nembutal® (Abbott) anaesthesia during irradiation, and non-irradiated controls were similarly anaesthetized. Some of the animals received a whole body irradiation with a single lethal dose of 900 R. Some of the animals carrying plasma cell tumours were similarly irradiated with their tumours shielded by a 3 mm thick lead plate.

The germ-free mice submitted to whole body irradiation received a single supralethal dose of 2000 R (mid-line air dose) while being constrained in a perforated plexiglass container. The irradiation factors were: 260 kV; 1 mm Cu+1 mm Al; h.v.l. 2 mm Cu; anti-cathode-object distance, 60 cm; dose-rate, 66 R min.

Intravenous serum transfusion

All animals of the germ-free group were given one intravenous injection of 2 ml of either mouse serum or saline, administered over 1 minute by way of the tail vein. The serum was obtained from adult conventional C₃H mice with high levels of serum IgA, and was used within 1 hour after bleeding the donors, having been stored at room temperature during the interval.

Intestinal washings

Catheters were passed into the proximal duodenum and distal ileum of laparotom-

ized mice, under Nembutal® (Abbott) anaesthesia. The contents were flushed out of the bowel segment between the two sutured catheters by means of 20 ml of warm (37°) phosphate buffered saline containing trypsin inhibitor (Trasylo® Bayer), over a period of 20 minutes. The effluent was immediately centrifuged, dialysed for 24 hours against saline, in the cold, and lyophilized.

Measurement of immunoglobulin concentrations

All BALB/c mice were bled 12 hours prior to irradiation, and again on days 3, 6 and 9 after the procedure, the first three samples of blood being obtained from the retro-orbital sinus (about 0.05 ml per sample) and the final specimen by decapitation. The germ-free Swiss mice were bled only from the retro-orbital sinus. All sera were stored separately at -25°.

Immunoglobulin determinations were performed by the Mancini technique (Mancini, Carbonara and Heremans, 1965) using antisera monospecific for mouse IgA or IgM, respectively (Bazin and Malet, 1969).

RESULTS

FATE OF IgA SECRETED BY A GRAFTED PLASMA CELL TUMOUR (TABLE 1)

Male BALB/c mice were separated into two lots, one of which (Groups A, C and D) received a subcutaneous graft of 1-2 mm³ of syngeneic S-121 plasma cell tumour secreting an IgA myeloma protein; the other lot (Group B) served as controls when the grafted tumours had reached a diameter of about 2 cm. A selection was made, discarding those

TABLE 1

CHANGES IN SERUM IgA AND IgM LEVELS IN MICE WITH IgA-SECRETING PLASMA CELL TUMOURS AND IRRADIATED WITH OR WITHOUT THE TUMOUR PROTECTED

Group	Animals	No. of animals	X-Ray dose	Ig	Serum immunoglobulin concentration (per cent of preirradiation level)			
					Preirradiation	Day 3	Day 6	Day 9
A	Tumour-bearing controls	6	None	IgA	13.23 ± 1.47* (100)†	11.45 ± 1.45 (88 ± 11)	13.32 ± 1.42 (103 ± 10)	17.84 ± 0.88 (140 ± 11)
				IgM	2.63 ± 0.47 (100)	3.52 ± 0.50 (114 ± 8)	2.53 ± 0.36 (105 ± 22)	2.17 ± 0.26 (86 ± 7)
B	Healthy controls	16	900 R	IgA	0.89 ± 0.08 (100)	0.55 ± 0.05 (64 ± 3)	0.38 ± 0.04 (43 ± 3)	0.19 ± 0.01 (26 ± 3)
				IgM	6.09 ± 0.39 (100)	5.09 ± 0.18 (86 ± 4)	4.52 ± 0.20 (76 ± 5)	2.61 ± 0.19 (45 ± 4)
C	Tumour-bearing mice irradiated including tumours	12	900 R	IgA	13.63 ± 2.25 (100)	6.36 ± 1.07 (57 ± 12)	2.09 ± 0.20 (20 ± 3)	0.81 ± 0.12 (9 ± 2)
				IgM	3.75 ± 0.42 (100)	3.67 ± 0.28 (95 ± 7)	2.82 ± 0.19 (82 ± 8)	2.35 ± 0.25 (71 ± 11)
D	Tumour-bearing mice irradiated excluding tumours	9	900 R	IgA	12.59 ± 2.25 (100)	13.40 ± 1.84 (107 ± 6)	13.41 ± 1.50 (112 ± 7)	12.39 ± 1.35 (103 ± 7)
				IgM	3.02 ± 0.33 (100)	2.73 ± 0.25 (97 ± 6)	2.30 ± 0.23 (82 ± 6)	1.96 ± 0.23 (70 ± 6)

* Serum concentrations of IgM and IgG are indicated as means ± SD (mg/ml).

† Mean per cent of base-line level ± SD.

animals which appeared in poor health or presented ulcerative skin lesions over the graft. In all, twenty-seven tumour-bearing mice were retained for the experiment and were submitted to a test bleed at the same time as sixteen control mice without tumours.

All the controls and twenty-one of the tumour-bearing mice were given a 900 R total body dose of X-rays. However, in nine of the tumour-bearing animals (Group D), the plasma growth was shielded from irradiation, as described under Methods.

The serum levels of IgM and IgA in the different groups of irradiated and sham-irradiated mice 12 hours prior to the experiment and 3, 6 and 9 days thereafter are indicated in Table 1. The base-line level of IgM was rather high in all animals in comparison to other published data (Bazin and Micklem, 1967; Bazin and Malet, 1969; Barth, McLaughlin and Fahey, 1965; Warner and Wistar, 1968).

The usual sharp decrease in serum IgA levels was observed in the conventional BALB/c mice (Group B, Table 1) following whole-body irradiation with 900 R. By the 9th day, their serum IgA level had fallen to 26 per cent of the baseline level, whereas the concentration of IgM in the serum had only been halved by that time.

The secretory activity of the tumour grafts raised the serum IgA levels to more than ten times the base-line values of the controls (Group A, C and D, Table 1). Whole-body irradiation including the tumours (Group C) produced a precipitous fall in serum IgA, exceeding in relative importance that observed in healthy irradiated mice. In contrast, when the tumours were protected from irradiation, the dose of 900 R did not in the least affect the serum IgA levels (Group D), whereas the concentration of IgM declined in a manner similar to that observed in grafted animals whose tumours had been irradiated.

INTESTINAL EXCRETION OF TRANSFUSED IgA AND IgM IN IRRADIATED GERM-FREE MICE (TABLE 2)

Twenty-four germ-free Swiss mice were removed from their isolators and separated into four groups of six animals each. After an initial control bleeding, the animals of Groups E and F were given an X-ray dose of 2000 R, whereas Groups G and H served as non-irradiated controls. The animals of Groups E, F and G received an intravenous injection of 2 ml of normal mouse serum on the 24th or 48th hour after the initial blood sampling (i.e. 12 or 36 hours after irradiation), whereas Group H received 2 ml of saline instead. 4-10 hours later, all animals were bled again from the retro-orbital sinus. They were then anaesthetized, and their intestinal contents were flushed out as described under Methods.

The results of this experiment are set out in Table 2. As expected, the serum of these germ-free animals contained only small amounts of IgM and hardly any IgA prior to the intravenous infusion of conventional mouse serum. The control mice of Group H, which had merely been bled and injected with saline, exhibited some decrease of the IgM levels during the 10 hour period of the experiment. In these controls, the intestinal fluid was found to contain essentially no IgM besides small amounts (0.028 ± 0.008 mg) of IgA. Intravenous injection of normal mouse serum resulted in a rise of serum IgA and serum IgM to well within the normal range (Group G), without causing any IgM or additional IgA to appear in the intestinal contents. Irradiation of germ-free mice injected with normal mouse serum did not significantly alter the decline in the serum levels of the transfused IgM or IgA (Groups E and F), as compared to their behaviour in the non-irradiated controls (Group G). Nor was there any difference in the intestinal excretion of IgM or IgA between these various groups.

TABLE 2
 INTESTINAL EXCRETION OF TRANSFUSED IgA AND IgM IN IRRADIATED MICE AND THEIR CONTROLS

Group	No. of animals	Irradiation dose (R)	Time lapse between irradiation and i.v. injection	I.V. Injection (2ml)	Ig	Serum level prior to injection (mg/ml)	Serum level 4 hours after injection (mg/ml)	Serum level 10 hours after injection (mg/ml)	Amount of immunoglobulins recovered from intestinal contents (mg)
E	6	2000	12 hours	Normal mouse serum	IgA	0.013 ± 0.006	0.307 ± 0.032	0.261 ± 0.023	0.023 ± 0.005
					IgM	0.94 ± 0.23	3.65 ± 0.32	3.60 ± 0.41	0
F	6	2000	36 hours	Normal mouse serum	IgA	0.011 ± 0.003	0.538 ± 0.040	0.485 ± 0.056	0.032 ± 0.007
					IgM	0.41 ± 0.11	4.25 ± 0.27	4.16 ± 0.33	0
G	6	0	—	Normal mouse serum	IgA	0.013 ± 0.004	0.374 ± 0.043	0.366 ± 0.049	0.029 ± 0.006
					IgM	0.86 ± 0.26	3.39 ± 0.28	3.89 ± 0.45	0
H	6	0	—	Saline	IgA	0.010 ± 0.004	0.010 ± 0.004	0.008 ± 0.003	0.028 ± 0.008
					IgM	0.53 ± 0.08	0.50 ± 0.07	0.44 ± 0.09	0

DISCUSSION

The present study was undertaken with the aim of elucidating the mechanism by which total body irradiation causes a selective fall in the concentration of serum IgA in the mouse. More specifically, confirmation was sought of the interpretation suggested by turnover data (Bazin and Malet, 1969), that this phenomenon is due to a failure in the renewal of the circulating IgA pool, rather than to a selective loss or hypercatabolism of this protein.

In order to restrict the number of metabolic variables, two systems were devised in which the source feeding the circulating IgA pool was inaccessible to damaging action of the X-rays. In this way, any selective fall in serum IgA levels occurring after irradiation should entirely be due to losses from the plasma pool. In the first type of experiments, normal mice were supplied with a huge excess of circulating IgA secreted by a grafted plasma cell tumour, this source of production being sheltered from the action of X-rays by means of a shield of lead. In the second type of experiment, severely hypogammaglobulinaemic germ-free mice, having practically no IgA of their own, were provided with IgA synthesized by conventional donor animals, by way of a whole serum transfusion.

The experiment on mice carrying IgA-secreting plasma cell tumours demonstrates that irradiation does not affect the catabolic decline of myeloma IgA, confirming the results of previous studies with tracer doses of ^{131}I -labelled myeloma IgA (Bazin and Malet, 1969). The grafted tumours themselves were very sensitive to X-ray damage, as shown by the extreme decline of serum IgA levels following irradiation in the animals of Group C (Table 1). With the tumours protected, however, there occurred no change in the concentration of the circulating myeloma IgA.

The germfree animals transfused with normal IgA-containing mouse serum received a dose of X-rays (2000 R) falling well within the supralethal range. This high dosage was chosen in order to inflict severe lesions upon the intestinal epithelium which, in germ-free mice, is known to be rather less sensitive to irradiation than in conventional animals (Van Bekkum, 1968). The latter finding has been related to the fact that the rate of renewal of the intestinal epithelium is only half as fast in germ-free mice as compared to conventional controls (Wilson, Bealmer and Matsuzawa, 1968). Correspondingly, the mean survival time of given supralethal doses of X-rays which cause the intestinal type of death (Quastler, 1956) has been found to be 7 days in germ-free animals against $3\frac{1}{2}$ days in conventional controls.

The transfusion procedure had evidently been successful in raising the serum IgA concentration in the germ-free mice to near-normal levels, as can be seen from a comparison between Groups E and F on one hand, with the control group H on the other hand (Table 2). In conventional mice, a single dose of 2000 R has previously been shown (Bazin and Doria, 1970) to cause a selective fall in serum IgA which is already significant by the 24th hour and becomes conspicuous by the 48th hour (See also Group B, Table 1). In contrast in the present experiments the germ-free transfused mice experienced no significant decline in the concentration of their serum IgA of donor origin after a similar irradiation procedure over the 10-hour observation period (Table 2, E and F). The behaviour of IgA in these animals was in fact not different from that of IgM. The latter immunoglobulin had been chosen as a point of comparison because turnover studies (Bazin and Malet, 1969) had shown that the half-lives of IgA and IgM in the mouse are about the same, i.e. 12 hours. The observed declines in the serum levels of IgA and IgM

in the irradiated animals of Groups E and F were in fact unexpectedly small, considering the high rates of catabolism of these two proteins. Similarly abnormally slow rates of disappearance of IgA and IgM were noted in the non-irradiated germ-free mice that had been infused with normal mouse serum. This would suggest that changes in the distribution of body fluids, associated with the infusion of large volumes of fluid were responsible for the abnormally slow metabolic decline in serum IgA levels in transfused germ-free mice, in contrast to what is observed in irradiated conventional animals.

In none of the four groups of animals in this second experiment could any measurable intestinal excretion of IgM be observed. The intestinal contents of germ-free mice infused with saline contained about 0.028 mg of IgA (Group H, Table 2). This amount of intestinal IgA was not increased by raising the serum IgA level toward normal values by means of a serum transfusion (Group G), nor by irradiation of animals whose serum IgA levels had been increased by the same procedure (Groups E and F). It is therefore clear that the amount of IgA recovered from the intestinal contents does not directly depend on the concentration of IgA prevailing in the serum. It is also evident that, whatever losses of plasma proteins into the gut may be caused by the irradiation procedure, such losses do not selectively affect the serum IgA level.

Hence, provided the source which supplies IgA to the serum pool is protected from the action of X-rays, there will occur no selective disturbance in the further metabolism of this serum immunoglobulin. In particular, the data presented here confirm the interpretation (Bazin, Maldague and Heremans, 1970) that X-rays do not cause any hypercatabolism or excessive digestive loss of IgA into the digestive tract. It will be shown elsewhere (Bazin, Maldague, Schonne, Crabbé, Bauldon and Heremans, 1971) that the specific metabolic lesion affecting IgA after irradiation resides in an interference with the pathway by which IgA synthesized in the bowel wall is fed into the plasma pool.

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The Metabolism of Different Immunoglobulin Classes in Irradiated Mice

V. CONTRIBUTION OF THE GUT TO SERUM IgA LEVELS IN NORMAL AND IRRADIATED MICE

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Summary. It was demonstrated in earlier publications that the acute and selective fall in serum IgA levels in irradiated mice was due to a specific failure to renew the plasma pool of this immunoglobulin. In the present work, it is shown that irradiation causes some reduction in the rate of biosynthesis of IgA, but that this phenomenon only partly accounts for the observed disturbance in the metabolism of IgA. This conclusion is based both on direct measurements on the rate of biosynthesis *in vitro* of IgA by the isolated small bowel wall of irradiated and normal C₃H mice, and on immunohistological studies and plasma cell counts on the gut, spleen and lymph nodes of these animals.

On the other hand, it is shown that irradiated mice continue to excrete approximately normal amounts of IgA into their intestinal contents. The conclusion is that irradiation of the gut causes a rechannelling in the output of IgA synthesized by the plasma cells of the lamina propria, the major part being diverted to the intestinal lumen instead of being fed into the circulating pool of serum IgA.

INTRODUCTION

It has previously been demonstrated that the very marked decrease in the concentration of immunoglobulin IgA in the serum of mice exposed to a whole-body dose of X-rays is not caused by excessive catabolic or intestinal losses of this protein. This was first inferred from the absence of changes in the metabolic half-life of ¹²⁵I-labelled myeloma IgA injected prior to irradiation (Bazin and Malet, 1969). The conclusion was strengthened by the recent demonstration (Bazin, Levi and Heremans, 1970) that lethal or supralethal X-irradiation was without significant effect on the fate of IgA synthesized by a grafted tumour, protected from the X-rays, or of IgA obtained from normal donor mice and infused into hypogammaglobulinaemic germ-free mice.

The logical implication of these negative findings was that the metabolic disturbance affecting IgA in irradiated animals resided in the mechanism responsible for the renewal of the circulating pool of this immunoglobulin. The present study is concerned with a search for positive arguments in favour of this view. The investigation was greatly facilitated by the previous demonstration (Bazin, Maldague and Heremans, 1970) that the gut was closely implicated in this metabolic lesion, since the selective fall in serum IgA did

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not occur when this organ was excluded from the action of X-rays, whereas the phenomenon occurred in full strength when the bowel alone was exposed to irradiation.

It will be demonstrated here that the rate of biosynthesis of IgA in the gut wall is only moderately affected by X-irradiation, and that the major cause of depletion of the serum IgA pool resides in the fact that much of the intestinal production of this immunoglobulin is diverted from its normal pathway of secretion into the serum to the benefit of abnormal excretion into the gut lumen.

MATERIALS AND METHODS

Animals

All experiments were carried out on C3H mice, aged 3 months, bred at the University of Louvain. Throughout the observation period the animals were allowed free access to standard food pellets and water.

Irradiation

A single dose of 700 R (Maxitron, General Electric, 250 KV, 1 Al+0.25 Cu filters, dose rate: 45 R/min) was administered to the mice while they were being constrained in Plexiglass boxes.

Antisera

The purification of mouse immunoglobulins and the preparation of the corresponding specific antisera in rabbits have been described elsewhere (Bazin 1966; Bazin and Malet, 1969). Each of these antisera produced a single precipitin line when tested against whole mouse serum in Ouchterlony plates or by immunoelectrophoretic assay.

Immunofluorescence

Antisera were labelled with fluorescein isothiocyanate according to published methods (Crabbé, Bazin, Eyssen and Heremans, 1968; Crabbé, Nash, Bazin, Eyssen and Heremans, 1969). These reagents were used without special absorption, as their specificity had been found to be satisfactory.

The animals were bled and their spleens, mesenteric, axillary and inguinal lymph nodes as well as a sample of the duodenum and ileum were snap-frozen with dry ice and processed to 3–5 μ sections. The further processing of the preparations was carried out as already described (Crabbé, Nash, Bazin, Eyssen and Heremans, 1970).

In counting fluorescent cells, an effort was made to reduce sampling errors by examining, for each class of immunoglobulins, at least four sections obtained from three different portions of each specimen. The specificity of the fluorescence was controlled by incubating some sections with fluorescent antisera previously absorbed with whole mouse serum until all precipitating antibodies had been removed, as judged by immunodiffusion assays.

Biosynthesis of IgA by mouse gut wall in vitro

(a) *Extracellular IgA in individual bowel cultures.* Immediately after a test bleeding, normal or irradiated mice were sacrificed by decapitation. The small bowel, including the duodenum and terminal ileum, was removed, rapidly flushed out with 20 ml of Eagle Minimum Medium (Serva, Heidelberg, Germany) containing 500 IU of penicillin and 500 mg of streptomycin per ml, and minced to minute fragments (of about 1 mm³) by

means of scissors, all operations being carried out at room temperature. The mince from each bowel was suspended in 20 ml of Eagle Minimum Medium to which 350 IU of penicillin, 250 mg of streptomycin, and 1 mg of human IgA had been added. The inclusion of human IgA was intended as an internal control of proteolysis; the protein used for this purpose had been purified from a human myeloma serum and found to sediment as a single 7S peak in the analytical ultracentrifuge.

Each culture mixture was incubated at 37° in 250 ml Erlenmeyer flasks, under gentle rocking. After 1 hour, 5 ml of culture fluid free of gross tissue debris was withdrawn, and stored at -20° after clarification by centrifugation.

The cultures were continued under the same conditions for another 24 hours, after which the fluid was clarified by centrifugation and frozen at -20°.

The 1-hour and the 25-hour culture fluids were then lyophilized, reconstituted with distilled water to final volumes of 1 ml and 2 ml respectively, and used for the determination of mouse IgA and human IgA as described below.

(b) *Intra- and extracellular IgA in pooled cultures.* The conditions of tissue culture in this experiment were the same as in the preceding experiment, except that twelve minced intestines were suspended in 240 ml of culture medium, contained in a 1 litre Erlenmeyer flask.

After 1 hour of incubation, 120 ml of the culture mixture, including the equivalent amount of tissue, were removed and frozen at -20°. Another 30 ml of culture fluid, without tissue, were also removed and stored at -20° after clarification by centrifugation. The remaining 90 ml of culture mixture was centrifuged and the supernatant stored at -20°, whereas the sediment was added to the remaining mixture, which was frozen *in toto*, without separation of cells and culture medium.

The samples containing tissue in addition to culture medium were repeatedly frozen and thawed before clarification by centrifugation. Ultimately, all samples were lyophilized and reconstituted with distilled water to small volumes, prior to the quantitative determination of their mouse and human IgA contents.

Intestinal perfusion

One day after a test bleeding, mice were irradiated with a whole-body dose of 700 R. One to 6 days later, the irradiated animals and their sham-irradiated controls were anaesthetized by an intraperitoneal injection of 1 ml of 1 per cent Rectanol® (Laboratoire Robert & Carrière, Paris) in saline, after another test bleeding. A rubber catheter (2 mm diameter) was inserted into the proximal part of the duodenum and sutured to the gut wall by means of silk. Another catheter (2 mm diameter) was affixed in similar fashion in the lumen of the terminal ileum. A total of 400 ml of phosphate buffered saline containing 100,000 IU of Trasylol® (Bayer, Leverkusen, W. Germany) intended as a trypsin inhibitor, was perfused through the small bowel over a period of 8 hours. The effluent was collected into ice-cold 1 M acetate buffer of pH 3.5. For each mouse, four successive 2-hour perfusates were collected, concentrated in the cold by dialysis *in vacuo*, to a final volume of 1-2 ml, and used as such for the determination of IgA.

Density gradient ultracentrifugation

A density gradient of sucrose (10-35 per cent) in borate buffered saline (pH 8.4) was employed. The protein samples were applied on top of the gradient. The SW 39 rotor of the Spinco Model L ultracentrifuge was run for 18-20 hours at 35,000 rev/min at 4°.

after which the tubes were punctured and drained from the bottom in 30–40 ten-drop fractions. Estimates of molecular size of mouse IgA and human IgA from different origins were obtained, using samples of mouse serum, 19S α_2 M, purified milk 11S IgA, purified human 7S IgG, purified human 7S myeloma serum IgA, human 5.5S transferrin and purified horse 2.6S immunoglobulin L chains as markers for the different molecular size regions. The sedimentation coefficients of the marker proteins were verified in the Spinco model E analytical ultracentrifuge.

Morphological studies

For electron microscopic studies, 2 cm-long segments of ileum or jejunum were carefully dissected and summarily fixed by gentle perfusion with 10 ml of a 2 per cent solution of glutaraldehyde in isotonic phosphate buffer of pH 7.4. Small fragments were post-fixed by immersion in the same glutaraldehyde solution for 24–48 hours, followed by additional fixation with osmium for 1 hour. Epon-embedded ultrathin sections were stained with uranyl acetate and lead citrate.

For optical microscopic studies, 1 mm thick transverse sections of ileal or jejunal tissue pre-fixed with glutaraldehyde and post-fixed with osmium as described above, were embedded in Epon and processed to 1 μ thickness by means of a Reichert Ultramicrotome. These sections were stained with a 1 per cent aqueous solution of toluidine blue for $\frac{1}{2}$ –1 hour.

Measurements of protein concentrations

Quantitative determinations of mouse immunoglobulins were performed by the Mancini technique (Mancini, Carbonara and Heremans, 1965) using antisera monospecific for mouse IgA, IgM, IgG₁ and IgG_{2a}, respectively (Bazin, 1966; Bazin and Malet, 1969).

The same technique was used for the quantitation of the proteins used as molecular size markers.

RESULTS

EFFECTS OF X-RAYS ON THE PLASMA CELLS OF DIFFERENT CLASSES IN VARIOUS TISSUES (TABLE 1)

In non-irradiated control mice the counts of plasma cells containing the different classes of mouse immunoglobulins, IgM, IgA, IgG₁ and IgG_{2a}, were in substantial agreement with previous findings (Crabbé *et al.*, 1970). In particular, the plasma cell population of the lamina propria of the duodenum and ileum was composed of an overwhelming majority of cells staining with anti-IgA (Table 1 and Fig. 1a).

After irradiation by 700 R, a moderate though significant reduction in plasma cell counts of all four immunoglobulin classes was observed in the intestinal lamina propria, but no measurable changes appeared to occur in the spleen and peripheral lymph nodes. The lymphoid tissue of the mesenteric lymph node shared some of the radiosensitivity of the lamina propria, although its overall response was more akin to that of the spleen. The decrease in plasma cell counts in the bowel wall became first noticeable towards the 2nd or 3rd day after irradiation and some tendency towards restoration was observed after 1 week.

In addition to these quantitative changes, the immunohistological appearance of the affected tissues conveyed the impression that the staining of the plasma cells with fluorescent antisera was less intense and more diffuse than usual (Fig. 1A and B).



FIG. 1. IgA-containing plasma cells in normal and irradiated mouse intestine. A, Jejunal mucosa from a normal control. B, Jejunal mucosa from a mouse, 4 days after 700 R whole body irradiation.

TABLE I
EFFECTS OF X-RAYS ON PLASMA CELL COUNTS

Time after irradiation	Controls	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
No. of mice	8	1	1	1	2	2	2	2	1	1
Intestine: Duodenum IgM	+	+	+	+	+	+	+	+	+	+
IgA	-	+	+	+	+	+	+	+	+	-
IgG ₁	+	+	-	-	-	-	-	+	+	+
IgG _{2a}	+	+	-	-	+	+	NT	-	-	-
Ileum IgM	-	-	+	-	+	+	+	+	+	+
IgA	+	+	+	+	+	+	+	+	+	+
IgG ₁	+	+	-	-	+	+	-	+	+	+
IgG _{2a}	+	+	-	-	-	-	NT	-	-	-
Mesenteric lymph nodes: IgM	+	+	+	+	+	+	+	+	+	+
IgA	+	+	+	+	+	+	+	+	+	+
IgG ₁	+	+	+	+	+	+	+	+	+	NT
IgG _{2a}	+	+	+	+	+	+	NT	+	+	NT

BIOSYNTHESIS OF IgA BY TISSUE CULTURES OF NORMAL AND IRRADIATED GUT (TABLE 2)

As expected, the decline in serum immunoglobulin levels in this series of irradiated mice was more pronounced in the case of IgA than with the other classes of immunoglobulins.

The IgA-synthesizing capacity of the tissue cultures of the bowel wall fragments was estimated from the net gain in IgA in the culture medium between the 1st and the 25th hour of incubation. In the cultures of non-irradiated mouse gut, this increase averaged 6.55 mg over the 24-hour period, compared to a total amount of 2.80 mg of IgA found at the onset of the observation period, i.e. after 1 hour of incubation. Both the amount of IgA present after 1 hour and the net increase in IgA during the ensuing 24-hour culture period were significantly lower in the irradiated animals than in the controls, the former quantity being reduced to about one-half and the second to about $\frac{2}{3}$ – $\frac{3}{4}$ of the values found in non-irradiated material.

To interpret the significance of net gain in IgA in such cultures, it is necessary to take into account a number of factors other than biosynthetic activity.

Firstly, one must reckon with the possibility that some of the IgA synthesized by the culture or present in the medium at the onset of the experiment may become degraded by proteolysis during the 24-hour period of incubation at 37°. Not only may proteolytic enzymes be expected to be produced by the intestinal mucosa, but one must also consider the possibility of proteolytic degradation by the cathepsins set free from cells which die during the observation period. The effects of proteolysis on the determination of the amount of IgA synthesized during the culture period may in turn be manifold. On one hand, any material sufficiently degraded to abolish its precipitation with the antiserum will escape detection; on the other hand, incomplete degradation might reduce molecules of mouse IgA to the size of their Fc fragments (Askonas and Fahey, 1962) which, on account of their fast diffusion may give rise in the Mancini assay to precipitin rings of excessive size (Heremans, 1970), leading to an overestimation of the amount of immunoglobulin actually synthesized.

Using human myeloma IgA as an internal control, it was indeed found in our cultures that about 60 per cent of this material became antigenically inactivated—and therefore presumably was destroyed—during the 24-hour incubation of the culture of normal mouse bowel. In irradiated animals, the amounts of human IgA lost under similar conditions were not significantly different from this normal control value, as judged by appropriate *t*-tests (Table 2).

An additional check on the possible incidence of proteolysis on the validity of the immunochemical quantitation of human or mouse IgA was provided by a direct evaluation of the molecular size distribution of these proteins in the supernatants of the cultures. In the density gradient, added human 7S IgA showed no evidence of degradation to antigenically active products smaller than 7S (Fig. 2, b–d). The IgA in normal mouse serum sedimented slightly slower than the human 11S marker, with little evidence of inhomogeneity (Fig. 2a).

After the 1st hour of incubation, much of the IgA present in the culture medium sedimented distinctly faster than 11S, with smaller amounts being present in the range characteristic of mouse serum IgA (Fig. 2b), and occasionally, also in the 7S range. There was no consistent difference between the IgA size distributions, after 1 hour, in the cultures from irradiated animals compared to those from the controls, except that in the

former the heavier forms of IgA tended to decrease in importance. The culture fluids analysed after 24 hours of incubation showed an entirely different distribution of mouse IgA, with the lighter forms predominating, both in the controls and in the irradiated animals (Fig. 2c, d).

TABLE 2
EFFECT OF X-RAYS ON SERUM IMMUNOGLOBULIN LEVELS AND IgA-SYNTHESIZING CAPACITY OF BOWEL WALL

Time after irradiation	Controls	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
No. of mice	12	5	5	5	5	5	5
	Serum immunoglobulin concentration						
IgM							
Pre-irradiation (mg/ml)	5.24 ± 0.36*	5.59 ± 0.11	5.82 ± 0.63	5.78 ± 0.32	5.73 ± 0.23	5.35 ± 0.25	5.47 ± 0.19
Post-irradiation (percentage of pre-irradiation)	112 ± 12	104 ± 8	91 ± 4	87 ± 5	78 ± 2	72 ± 6	77 ± 8
IgG ₁							
Pre-irradiation (mg/ml)	0.99 ± 0.07	1.19 ± 0.15	1.11 ± 0.10	0.78 ± 0.11	1.13 ± 0.07	0.93 ± 0.13	1.03 ± 0.04
Post-irradiation (percentage of pre-irradiation)	108 ± 10	102 ± 7	84 ± 7	112 ± 20	82 ± 7	85 ± 13	71 ± 3
IgA							
Pre-irradiation (mg/ml)	1.72 ± 0.23	1.75 ± 0.39	2.48 ± 0.23	1.74 ± 0.30	2.79 ± 0.27	1.70 ± 0.25	1.78 ± 0.22
Post-irradiation (percentage of pre-irradiation)	122 ± 19	87 ± 9	39 ± 3	62 ± 7	33 ± 5	57 ± 9	44 ± 5
	IgA-Synthesizing capacity of bowel wall cultures						
Mouse IgA in culture medium							
After 1 hour (mg)	2.80 ± 0.24	1.88 ± 0.16	1.69 ± 0.18	1.27 ± 0.12	1.40 ± 0.10	1.30 ± 0.88	1.36 ± 0.05
Net gain 24 hours later (mg)	6.55 ± 0.75	4.95 ± 0.86	3.74 ± 0.36	4.17 ± 0.27	3.64 ± 0.20	5.20 ± 1.05	4.00 ± 0.15
Net gain in 24 hours as percentage of net gain in controls	100	75	57	64	56	79	61
Human IgA percentage control degraded by culture 24 hours	58 ± 7	64 ± 9	76 ± 2	73 ± 5	49 ± 9	56 ± 8	34 ± 14

* Values are indicated as means ± SD.

On the whole, these density gradient analyses indicate that no significant amounts of material the size of IgA-Fc fragments were formed during the incubation, and that therefore, the quantitative estimates of IgA obtained by the Mancini method were not in error by excess.

Another factor to be taken into account is that the net increase in IgA in the culture medium over the 24-hour period of incubation need not necessarily be attributed to *de novo* biosynthesis alone; part of this IgA might represent preformed intracellular protein released during the incubation period, either by a process of active secretion or through

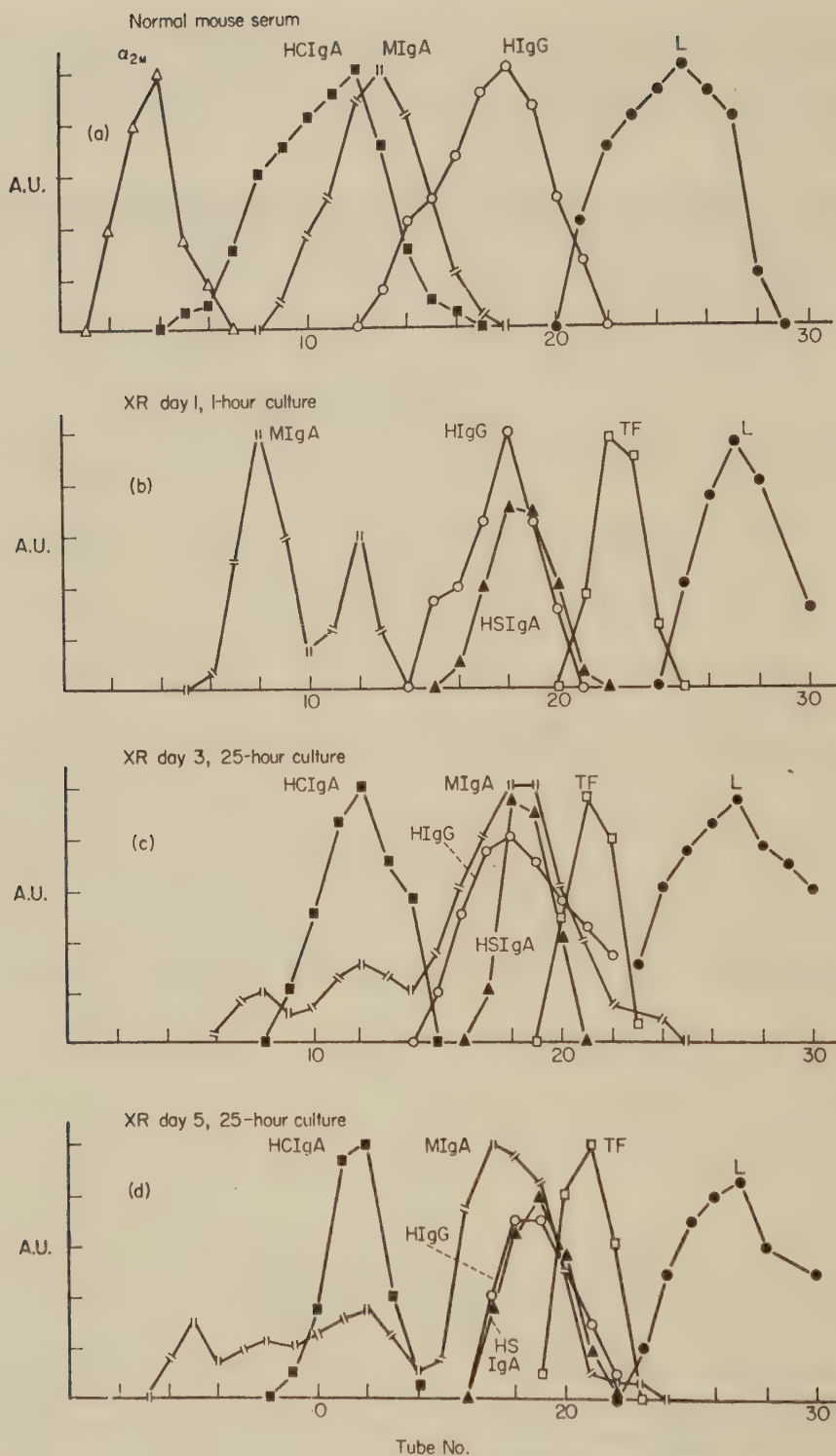


FIG. 2. Molecular size distribution of mouse IgA in tissue cultures of small bowel wall. Sucrose density gradient ultracentrifugation (sedimentation from right to left) of: (a) Normal mouse serum; (b-d) supernatants of cultures of intestine from irradiated mice. Abscissae, tube numbers; ordinates, arbitrary protein concentration units. Abbreviations: α_{2M} , mouse α_2 -macroglobulin (19S); TF, human transferrin (5.5S); L, light chains from horse IgG (2.6S); HClgA, purified IgA from human colostrum; HSIgA, human serum (myeloma) IgA (7S); MIgA, mouse IgA; HIgG, human IgG (7S); HIgG, human IgG (7S).

decay of the cells. To assess the participation of secretion or release of IgA, an experiment was devised in which intra- and extracellular IgA were titrated separately in the cultures, both after 1 hour and after an additional 24 hours of incubation. The results are set out in Table 3 and indicate that the net gain is practically entirely accounted for by newly synthesized IgA.

INTESTINAL EXCRETION OF IgA IN IRRADIATED MICE

In the series of animals submitted to intestinal perfusion following irradiation by 700 R, the usual pronounced fall in serum IgA levels was observed (Table 4). This phenomenon first became perceptible by the 3rd day and reached impressive proportions after 5 or 6 days.

In view of the broad physiological range of the serum IgA levels in normal mice (Table 4), the intestinal perfusion data are indicated both in absolute values and compared to the concentration of serum IgA in the individual groups prior to irradiation or perfusion.

TABLE 3
INTRA- AND EXTRACELLULAR IgA IN TISSUE CULTURES OF MOUSE GUT

Time of incubation	IgA in culture fluid = Extracellular IgA (mg)	IgA in culture fluid + cells (mg)	Difference = Intracellular IgA (mg)
1 hour	9.84	14.16	4.32
25 hours	18.00	21.84	3.84
Net gain	8.16	7.68	-0.48

The results indicate that the cumulative amounts of IgA recovered over an 8-hour period of intestinal perfusion were not significantly different in irradiated animals compared to controls. Nor were the recoveries of intestinal IgA markedly different at different time intervals after irradiation, except perhaps for the last day when the animals were so debilitated as to succumb during the perfusion. In each experiment, it was noted that the amounts recovered decreased sharply during the first 2-hour period, to remain more or less constant thereafter.

When expressed by the ratio of intestinal IgA *vs* serum IgA prior to irradiation, the data of Table 4 confirm the absence of significant changes after exposure to 700 R, except in the very early or very late stages of the observation when this ratio was somewhat decreased.

In contrast, the amounts of IgA recovered from the intestinal contents grew disproportionately large in comparison to the declining serum IgA levels measured just prior to the perfusion, during the days following irradiation, and this discrepancy, compared to the normal intestinal: serum IgA ratio, also became increasingly significant.

As in the tissue culture experiment, it was necessary to check the validity of the immunochemical quantitation method by verifying the molecular size distribution of the IgA titrated in the intestinal perfusion fluids. Owing to the limited amounts of IgA recovered after the 2nd hour of perfusion, these analyses had to be restricted to the initial sample of each series. In control mice, intestinal IgA sedimented in a rather heterogeneous manner, the bulk of the material being distinctly heavier than the 11S human marker IgA

TABLE 4
INTESTINAL EXCRETION OF IgA IN IRRADIATED MICE

Time after irradiation	Controls	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
No. of mice	13	3	3	6	7	4	2
Serum IgA levels							
Before irradiation (mg/ml)	—	4.45 ± 1.17*	2.35 ± 1.51	3.10 ± 0.55	2.79 ± 0.61	4.62 ± 0.23	4.09 ± 0.14
After irradiation just prior to intestinal perfusion (mg/ml)	—	—	—	—	—	—	—
Difference (per cent of pre-irradiation level)	—	99	117	40	41	18	12
Amounts of IgA recovered by perfusion of the gut							
Perfusion periods							
Hours 0-2 (mg)	0.44 ± 0.06	0.29 ± 0.06	0.29 ± 0.08	0.49 ± 0.25	0.44 ± 0.09	0.41 ± 0.07	0.18 ± 0.06
Hours 2-4 (mg)	0.16 ± 0.05	0.13 ± 0.02	0.03 ± 0.02	0.11 ± 0.03	0.11 ± 0.01	0.19 ± 0.02	0.06 ± 0.02
Hours 4-6 (mg)	0.09 ± 0.01	0.07 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.11 ± 0.01	0.16 ± 0.01	0.09 ± 0.02
Hours 6-8 (mg)	0.09 ± 0.01	0.03 ± 0.00	0.04 ± 0.04	0.06 ± 0.00	0.09 ± 0.01	0.16 ± 0.03	0.09 ± 0.02
Total amount (mg)	0.78	0.52	0.37	0.71	0.75	0.92	0.42
Intestinal IgA recovered over 8-hour period							
Ratio		Amount of IgA in 1 ml of serum					
Referred to serum IgA level prior to irradiation (Significance)	—	12.4 ± 1.7 $P < 0.02†$	43.0 ± 32.0 NS	27.3 ± 9.3 NS	45.1 ± 15.8 NS	19.9 ± 1.6 NS	10.3 ± 1.8 $< 0.05†$
Referred to serum IgA level after irradiation but just prior to perfusion (Significance)	30.0 ± 3.0 —	13.6 ± 2.6 $P < 0.05†$	37.2 ± 26.0 NS	66.6 ± 22.4 $P < 0.05†$	104.0 ± 37.3 $P < 0.02†$	145.4 ± 38.8 $P < 0.01†$	86.4 ± 13.6 $P < 0.01†$

* Values are indicated as means ± SD.
† Significantly lower than in controls.
‡ Significantly higher than in controls.
NS, Not significant.

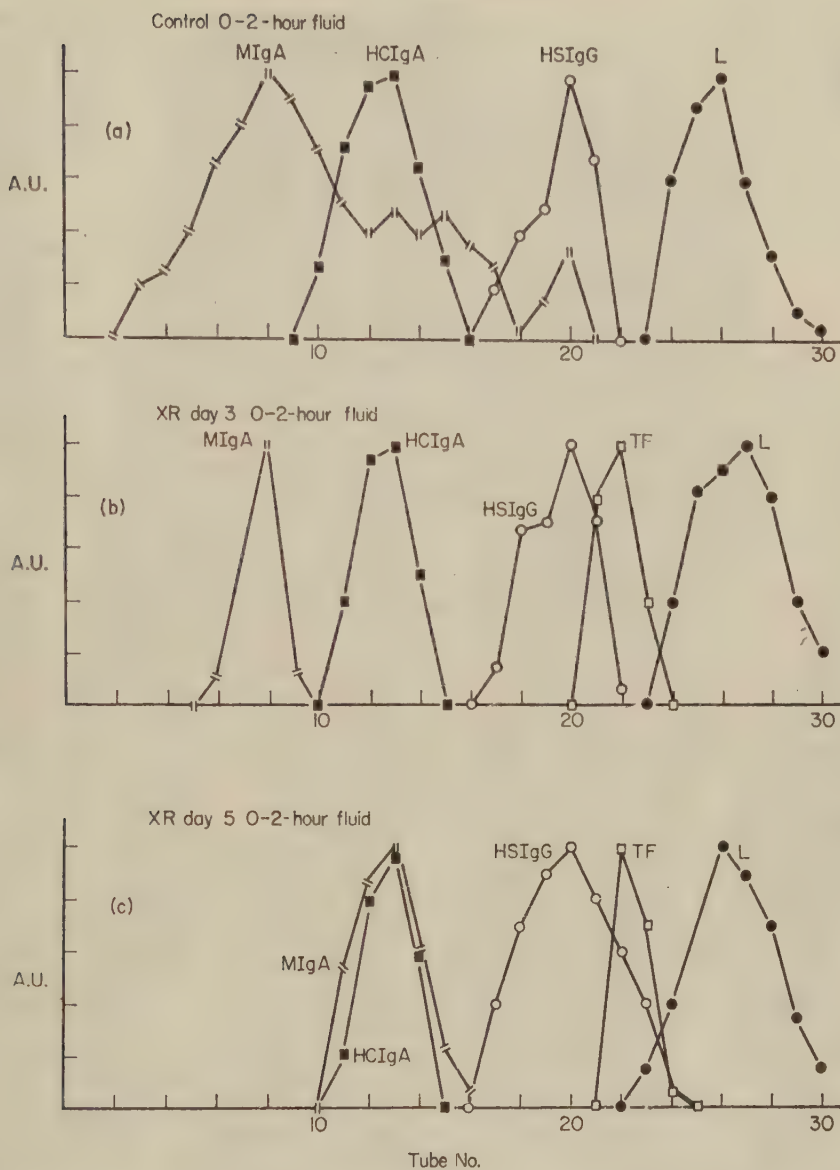


FIG. 3. Molecular size distribution of mouse IgA in perfusates of the bowel lumen; same conditions and indications as in Fig. 2.

(Fig. 3a). After irradiation, the major part of intestinal IgA continued to sediment together with or faster than the 11S markers, no material smaller than this size being present in significant amounts (Fig. 3b, c).

HISTOLOGICAL AND ELECTRON MICROSCOPIC STUDIES

A detailed description of the morphological lesions in the gut of irradiated mice will be presented elsewhere (Maldegue, Baudon, Hamels, Bazin and Heremans, in prep.) but the following findings will be summarized here, as they have an immediate bearing upon the pathogenesis of the metabolic lesions of IgA following irradiation.

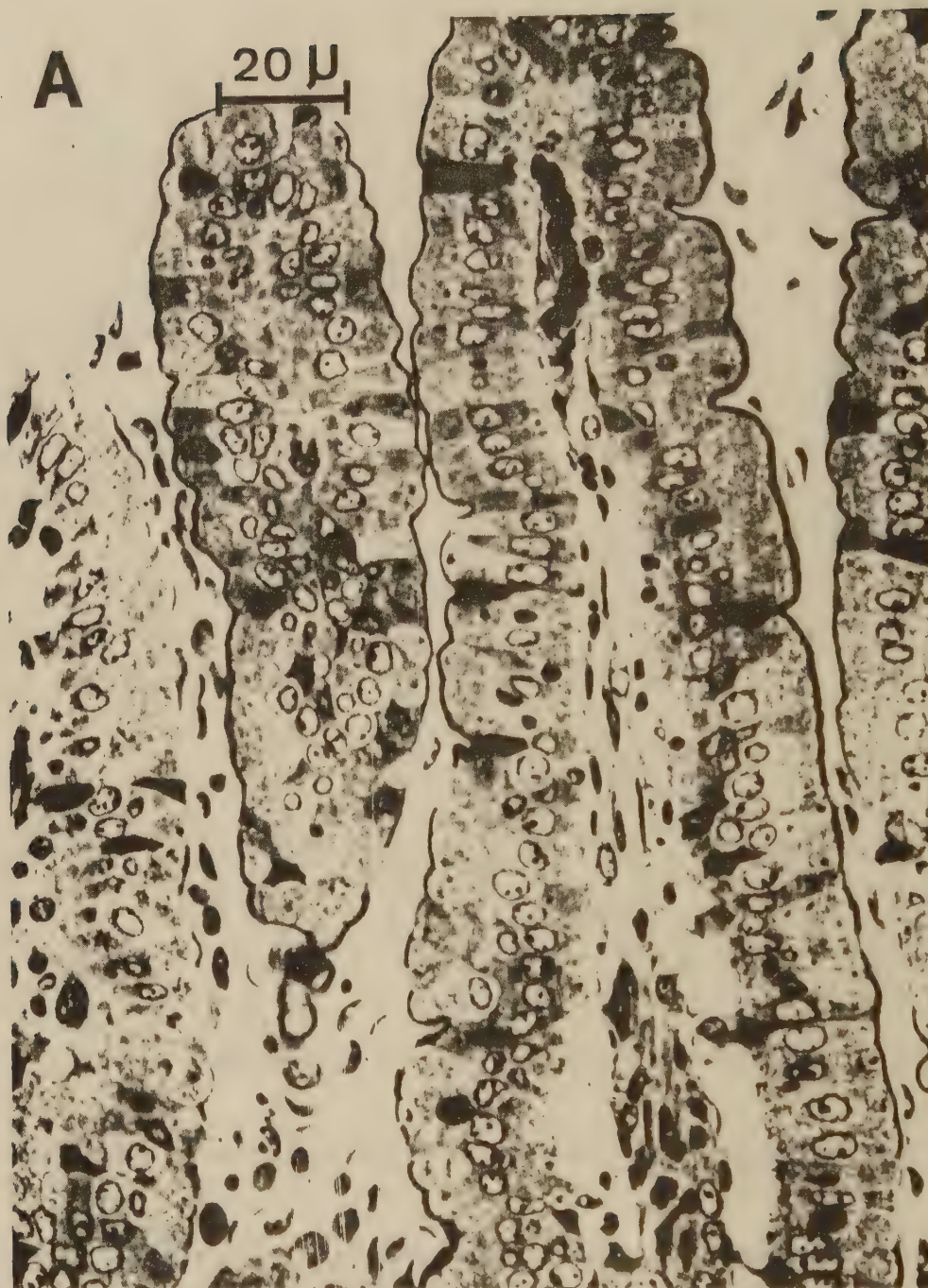


FIG. 4. Low-power view of normal and irradiated intestinal mucosa. Epon-embedded semi-thin sections ($1\ \mu$) stained with toluidine blue. A Non-irradiated jejunum; B Jejunum, 3rd day after 700 R; C Ileum, 3rd day after 700 R. Note shortening of villi and reduction in number of epithelial cells, without gross drastic discontinuities in the epithelial lining. Note also tendency of stroma to collapse.

X-ray damage resulted in a marked reduction in number and size of the epithelial cells (Fig. 4B and C), both factors causing a shortening of the villi. These abnormalities reached a maximum by the 3rd day after irradiation. They were associated with a great reduction in rough ergastoplasmic reticulum and a disappearance of the microvilli



FIG. 4B.

(Fig. 7). In the jejunum (Fig. 4B) the cytoplasm of the epithelial cells was found to be loaded with fat droplets. Some regeneration, as judged by mitotic activity, already started by the second day irradiation, but even on day 4 most of the newly formed ribosomes still occurred as free clusters (Fig. 8), similar to those in immature cells.

The intercellular junctions between adjoining epithelial cells were studied with particular care on account of their possible bearing on the excretion of IgA into lumen of the

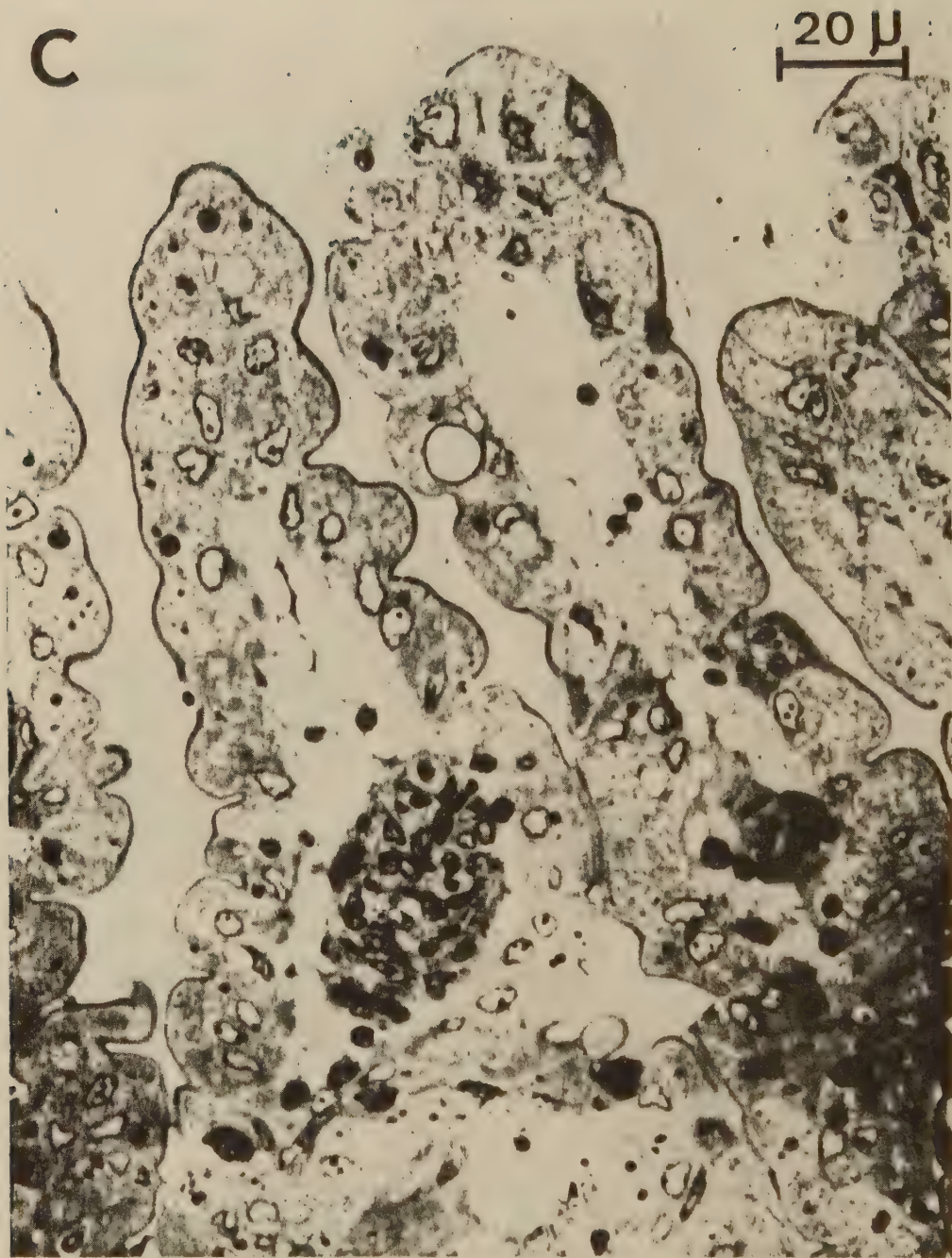


FIG. 4C.

gut. In this regard, the optical microscopic studies of the normal (Fig. 2A) and irradiated (Fig. 4B, C) epithelium provided no information, but the electron microscopic pictures disclosed an interesting finding. In the normal epithelium, the spaces separating adjoining cells were extremely narrow and of absolutely regular width, from the basement membrane up to the luminal border, being obliterated at places by desmosomes and closed, at the apical end, by a terminal bar (Fig. 5). 1–2 days after a lethal dose of X-rays, some widening of these intercellular spaces was noted, especially in the epithelium of the crypts

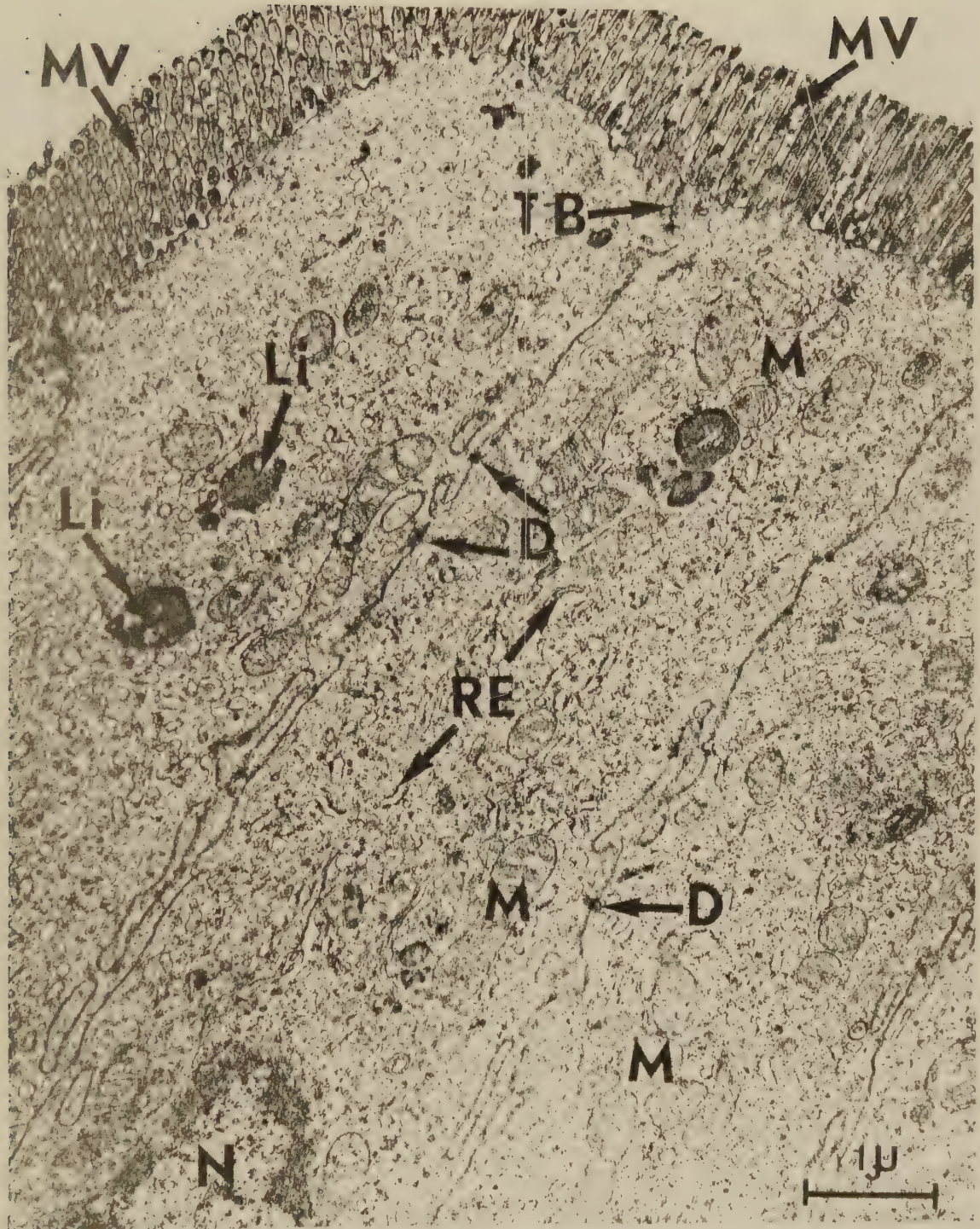


FIG. 5. Ultrastructure of cell junctions in normal jejunal epithelium. N, nucleus; M, mitochondria; Li, lipid droplets; MV, microvilli; RE, ribosome-studded endoplasmic reticulum. Note regularity and tightness of intricately folded intercellular spaces which are bridged by desmosomes (D) and terminal bars (TB).



FIG. 6. Epithelial cell junctions 2 days after irradiation. Note irregular widening of intercellular spaces (arrows) with normal persistence of desmosomes (D).



Fig. 7. Epithelial cell junctions 3 days after irradiation. Basement membrane (BM) and terminal bars (TB) remain intact but at many places the intercellular spaces have expanded to form lakes (L) providing almost patent communication between connective tissue space (CTS) and intestinal lumen (Lu).



FIG. 8. Epithelial cell junctions 4 days after irradiation. Despite evident signs of repair (hypertrophic nuclei, N; numerous free ribosomes, R), cell junctions still show some patent spaces (arrows) and are not folded (compare Fig. 5).



FIG. 9. Irradiated intestinal plasma cell (day 3). Note absence of significant alterations of cell organelles.

(Fig. 6). On the 3rd day after irradiation (Fig. 7), the intercellular spaces had become widely patent, creating a network of intercellular channels which freely abutted on the basement membrane. Desmosomes appeared to be relatively resistant to disjunction, and most of the bars were found to be intact. In no instance was gross sloughing of the epithelium observed and even the tips of the villi presented no discontinuities in excess of those observed in normal animals (Fig. 4). It was also noticed that during the repair phase the reestablishment of intercellular contacts progressed more slowly than the restoration of the cytoplasmic size and shape (Fig. 8). In many places desmosomes could be seen to interrupt areas of incomplete attachment of adjoining cells.

In confirmation of the immunohistological observations, it was found that the lamina propria tissue did not suffer a marked decrease in the numbers of its plasma cells. Many of the latter retained their normal ultrastructural appearance, even at the peak of radiation damage on the 3rd day (Fig. 9).

DISCUSSION

(1) *Effects of X-rays on the rate of biosynthesis of IgA*

As explained in the introduction, there exists a whole body of evidence pointing to a selective failure in the renewal of the pool of circulating IgA as being the mechanism responsible for the peculiar effects of X-rays on the serum levels of this class of immunoglobulins.

The most straightforward explanation would of course be that the biosynthesis of IgA is more sensitive to damage by X-rays than that of other immunoglobulin classes.

The protein-synthesizing machinery of cells in general is not reputed to be particularly affected by doses of ionizing radiation that would cause haematopoietic death if given on a whole-body level (Sarker, Devi and Hempelmann, 1961; Lipkin, Quastler and Muggia, 1963), and this seems true also in the special case of immunoglobulin-producing cells (Kennedy, Till, Siminovitch and McCulloch, 1965; Makinodan, Nettesheim, Morita and Chadwick, 1967; Reuter, Sassen and Kennes, 1967; Bazin, 1968; Sado, 1969). Indeed, whatever immunosuppressive effects may be credited to X-rays appear to rest chiefly on damage inflicted upon cell types involved in the earlier stages of the immune response (Kennedy *et al.*, 1965; Makinodan *et al.*, 1967). It must be stressed, however, that demonstrations of the relative radioresistance of immunoglobulin-synthesizing cells have been concerned with extraintestinal lymphoid tissue and with immunoglobulin classes other than IgA. That these reservations may be relevant is suggested by a report that the staining of the intestinal plasma cells of the guinea-pig with pyronin is decreased by whole-body X-irradiation with doses as low as 300 R (Ansari, Eder and Nägele, 1962). Similarly, ultrastructural lesions were described in the intestinal plasma cells of mice submitted to whole-body irradiation with lethal doses (Deby and Malkany, 1968), but neither in this nor in the preceding report was any comparative study on extraintestinal plasma cells included.

The work presented here (Table 1) indicates that a whole-body dose of 700 R caused a moderate reduction in numbers of the plasma cells in the small bowel wall, whereas no such effect was observed in the spleen or peripheral lymph nodes. It would also seem that this relative radiosensitivity of intestinal plasma cells is related to the particular environment to which they are exposed in the gut, rather than to their being specialized in the biosynthesis of the IgA class of immunoglobulins, since the rare cells producing IgM,

IgG₁ or IgG_{2a} appeared as much affected as those of the IgA type. One may venture the thought that toxic metabolites from damaged cells as well as massive admittance of bacterial endotoxins from the gut contents are involved in this increased vulnerability of plasma cells in an organ whose exquisite radiosensitivity first attracted attention 60 years ago (Regaud, Nogier and Lacassagne, 1912).

Since the majority of the intestinal plasma cells synthesize IgA (Crabbé *et al.*, 1968), and since in the dog, nearly 80 per cent of the circulating IgA is contributed by the gut (Vaerman and Heremans, 1970), it seemed tempting to ascribe the selective fall in serum IgA after irradiation to the relative radiosensitivity of the intestinal plasma cells. This hypothesis would also agree with the previous demonstration (Bazin *et al.*, 1970) that the gut is the site of the metabolic lesion affecting serum IgA in irradiated mice.

Nevertheless, the quantitative data provided by the biosynthesis experiments with isolated pieces of intestine indicate that the preceding explanation, though partially valid, cannot account for the major part of the fall in serum IgA levels following irradiation. The decrease in the rate of biosynthesis of IgA already suggested by the immunohistological studies, was confirmed, but the lowest activity observed (at day 4) still attained 56 per cent of the normal. On the average, the rate of synthesis of IgA by the gut was reduced by about one-third. This does not match the 50–90 per cent fall in serum IgA levels (Bazin and Micklem, 1967; Bazin, 1968; Bazin and Malet, 1969; Bazin *et al.*, 1970; Bazin and Doria, 1970).

2. Effects of X-rays on the fate of IgA produced by the gut

There is good evidence that the IgA synthesized by the plasma cells from the lamina propria is drained off in two opposite directions. Part of it reaches the blood stream *via* the mesenteric lymphatics, as demonstrated by comparative analyses of serum and mesenteric lymph in the dog (Vaerman and Heremans, 1970), as well as by the finding that X-ray damage to the gut caused a selective fall in serum IgA (Bazin *et al.*, 1970) without affecting the rate of disappearance of this protein from the serum (Bazin and Malet, 1970; Bazin *et al.*, 1970). On the other hand, the intestinal plasma cells of the mouse must be regarded as the major source of the IgA found in intestinal secretions, since only negligible amounts of serum IgA were found to pass into the intestinal contents (Crabbé *et al.*, 1968; Bazin *et al.*, 1970).

It was suggested in a preceding article (Bazin *et al.*, 1970) that the selective effects of X-rays on serum IgA might be explained by a shift in the distribution of IgA synthesized in the bowel wall, the proportion excreted increasing at the expense of the proportion supplied to the serum. This hypothesis seems to be substantiated by the findings reported here. It was found (Table 4) that the absolute amounts of IgA excreted into the lumen of the small bowel did not undergo any significant reduction following irradiation, although the total amounts synthesized (Table 2) did decrease by about one-third.

The lack of effects of X-rays on intestinal IgA is best brought out when the values found for the latter are compared to the serum IgA levels prior to irradiation, so as to cancel any variation due to individual differences in IgA-synthesizing capacities. The combined result of the invariance of the absolute intestinal IgA excretion rate and the fall in serum IgA following irradiation was a rise, peaking at day 5, of the *relative* amounts of intestinal IgA, as referred to the serum IgA level during the perfusion experiment (Table 4).

In conclusion, irradiation of the gut brings about a modest decrease in the IgA-synthesizing capacity of this organ, but the rate at which IgA is supplied to the intestinal

contents is not perceptibly affected. This can only mean that the contribution of the bowel wall to the pool of IgA in the blood stream must be affected very severely and the latter factor must in fact constitute the main reason for the observed selective fall in serum IgA levels.

The explanation for this shift in the distribution of IgA produced by the lamina propria is suggested by the morphological findings. The great radiosensitivity of the stem cells of the intestinal epithelium (Bloom and Bloom, 1954) is obviously responsible for the reduction in number of the cells coating the villi (Friedman, 1945; Knowlton and Hempelman, 1949; Weber, Craig and Friedman, 1951; Williams, De Long and Jarre, 1952; Williams, Toal, White and Carpenter, 1958), as well as for their flattening in shape and, indirectly, for the macroscopic shortening of the villi (Fig. 4B and C). The patency of the intercellular spaces after irradiation was cursorily mentioned by several authors (Quastler and Hampton, 1962; Detrick, Latta, Uphman and McCandles, 1963; Hugon, Maisin and Borgers, 1963, 1966), but its significance in the derangement of the function of the epithelial barrier does not seem to have been fully appreciated. The present work demonstrates that these communications between the interstitial spaces of the lamina propria and the intestinal lumen are a constant lesion after an X-ray dose of 700 R, and that leaks persist at a time when the more conspicuous cytoplasmic lesions are already largely repaired. There is little doubt that these abnormal communications provide the pathway by which the distribution of IgA synthesized in the lamina propria is rechannelled to the benefit of the intestinal contents and at the expense of the circulating pool of serum IgA.

An additional lesion which might play a role resides in the gross disorganization of the lacteal capillaries draining the interstitial lamina propria fluid (Fig. 4C), suggesting the possibility of obstruction along the pathway leading from this fluid to the bloodstream.

Light and electron microscopic studies of the irradiated mouse gut disclosed numerous patent intercellular junctions by which lamina propria fluid may escape into the intestinal lumen. It is suggested that this is the morphological substrate for the observed metabolic disturbance affecting IgA after irradiation.

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